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SERIAL IMPLANTATION OF ANTERIOR LOBES OF BOVINE AND HUMAN PITUITARY GLANDS INTO GUINEA-PIGS

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One of us and his collaborators [1] have shown that in a general way it is possible to distinguish three types of effects after implantation of the anterior lobe of the pituitary gland of cattle into the immature female guinea-pig, namely, (1) a follicle-destroying, or atresia-producing, action, due to a substance in the anterior lobe which we have designated atresin, (2) various types of luteinization of cells of the theca interna, as well as premature luteinization or maturation of the granulosa of not full-sized follicles, and (3) maturation of the granulosa of large follicles. In addition, there may occur an ingrowth of connective tissue and blood vessels into the granulosa of large, and also the granulosa of smaller, follicles. Rupture of large mature follicles may take place and be followed by formation of corpora lutea. These experimental interferences therefore call forth essentially pathologic processes in the ovary, some of which may be induced also by other procedures of a more generally injurious nature, such as underfeeding or irradiation.

Furthermore, they have shown that it is possible to modify these different effects which the bovine anterior lobe exerts by placing it, before implantation, in various solutions.[2] The latter prevent certain effects of the subsequently implanted anterior lobe from becoming mani-

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fest, while they favor others. Numerous solutions act specifically; while the majority tend to diminish or suppress the atresia-producing action and to promote the processes of luteinization, others, such as certain solutions of formaldehyde and urea, destroy not only the former but also the latter reactions, though they may allow the growth maturation effect to proceed. However, in addition, there were indications that even after implantation of the normal gland, it is possible to alter the equilibrium which exists between the atresia-producing and luteinizing effects, so that either one or the other may be favored, by varying the size of the piece implanted.

It seemed probable, moreover, that a certain sequence exists in the appearance of these various effects of the anterior lobe of the pituitary gland after implantation into the immature guinea-pigs, owing to a differential extraction of the substances to which these effects are due. In order to test this suggestion we implanted, serially, anterior lobes of the pituitary glands of cattle, each lobe having been cut into eight or sixteen small parts, into two or three guinea-pigs in succession. The pieces of a lobe were allowed to remain for a certain time in the first guinea-pig, and after they had had a chance to exert certain effects in this animal they were transplanted into a second guinea-pig and, in a number of cases, even into a third guinea-pig. In addition to the experiments with the anterior lobes of the pituitary glands of cattle, we made two experiments with the anterior lobes of human glands.

EXPERIMENTS

Group 1.—Seven experiments were made, in which the anterior lobes of the pituitary glands of cattle were used, each lobe being divided into four parts. The initial weights of the guinea-pigs varied between 170 and 210 Gm.; in most cases, between 175 and 185 Gm. In some instances the fourths of a lobe were implanted, one a day, into a first guinea-pig on four successive days; each implanted fourth was removed after it had remained in the animal for one day and was implanted into a second animal. Here it was allowed to remain until the fifth day, when examination of the effects of the implants on the animals took place. In other cases each piece implanted into a second guinea-pig was allowed to remain one day and then was implanted into a third animal, where the pieces were left for one, two, three and four days, respectively. In still other cases all four pieces were implanted into the first guinea-pig at the same time and allowed to remain for four days; they were then implanted, one piece daily, into a second guinea-pig and, in some cases, into a third guinea-pig. In each case the ovaries were examined in serial sections; also, the thyroid gland and, if necessary, the vagina and uterus were studied microscopically. In three of these seven serial experiments there was a gradual change from the atresia-producing

action, with or without processes of luteinization, in the first guinea-pig, to predominance of processes of maturation, in the second and third animals.

The results obtained in experiment 7 may serve as an example. Fourths of a bovine anterior lobe were implanted, one a day, into a guinea-pig; each piece was left for twenty-four hours and then reimplanted into another guinea-pig. First guinea-pig: Atresia-producing action predominated, but some pseudolutein bodies were seen. Second guinea-pig: Instead of atresia-producing action, large good follicles appeared; there were also early formation of pseudolutein bodies, growth of connective tissue into mature follicles and premature maturation of the granulosa of small follicles. Also, small interstitial gland bodies developed. Third guinea-pig: Large mature follicles and large follicles with net granulosa developed. There was moderate formation of interstitial gland in the medulla of the ovary, as well as some rudimentary interstitial gland bodies. There was, therefore, not only disappearance of the follicle-destroying, or atresia-producing action but marked diminution in luteinization in the third guinea-pig.

In the four other experiments, we found in the first guinea-pig predominance of atresia-producing action and in the second guinea-pig predominance of processes of luteinization in the theca interna, the granulosa or both. Experiment 3 may be cited. First guinea-pig, in which pieces were left only two days: There were early atresia-producing action and moderate formation of interstitial glands in the medulla. Second guinea-pig: Large follicles had developed, but their granulosa had degenerated; there were processes of luteinization in the theca interna and small interstitial gland bodies. There were also processes of luteinization in the granulosa and production of several pseudolutein bodies.

In the majority of these animals hypertrophy of the thyroid gland was not marked. In some cases there was noticeable decrease in the strength of the thyroid-stimulating action in the second and third guinea-pigs, but this decrease was covered up in some cases by the large quantity of anterior lobe acting in the second and third guinea-pigs as compared with the first animal.

Group 2.—This group consists of eight experiments; it differs from the previous group in that the implants from one whole anterior lobe were all made at the same time and the pieces were left for two, three or four days, not only in the first animal, but also in the animals in which they were implanted in succession. In two of these experiments the second guinea-pig died prematurely after one and a half and two days, respectively; in all the other experiments the pieces of gland were

transferred successively into three guinea-pigs, and the organs of these animals were examined on the fifth day after implantation. The average weight of these guinea-pigs was about the same as in the former group. Three experiments were carried out in June 1936, at the time when the summer heat had just begun, but the animals were still in good condition; the other five were carried out in October, when a very hot summer season was just ending. In the first serial guinea-pigs of the second group the atresia-producing action did not predominate to the same extent as in the previous group, in which one piece a day was implanted for four successive days; usually some follicles reached, therefore, a larger size; in addition, processes of luteinization of the theca interna and granulosa, consisting in luteinizing connective tissue atresia and formation of medullary interstitial gland, of interstitial gland bodies and of pseudolutein bodies and pseudocorpora lutea, were noticeable; only in one case were mature and maturing follicles found in addition to these pseudolutein structures. In two of the experiments belonging to the June group, maturation of large follicles became more prominent in the second and third guinea-pigs. The results were, then, in principle, similar to those obtained in the first group of experiments. In all the other experiments, in the course of succeeding transplantations the luteinizing and maturation effects were gradually lost and either atresia-producing effects predominated or the ovary returned to its normal state. These results were probably due to gradual extraction of the hormones in the course of the successive serial implantations. Also the thyroid hormone was much diminished or was lost entirely in the course of successive serial implantations.

Two experiments may be cited: 1. Experiment D (October 1936). Implantations were made every two days. First serial guinea-pigs: atresia-producing action predominated, but there was one very large follicle with partly preserved granulosa which became luteinized and which was invaded by connective tissue; this represented a rudimentary formation of pseudocorpus luteum. The thyroid showed marked hypertrophy. Second serial guinea-pig: There were large, good follicles with beginning maturation of granulosa. Some interstitial gland was noted in the medulla; otherwise there was the usual kind of follicles. The vagina was resting. The thyroid showed much hypertrophy, but the greater part of the colloid was left. Third serial guinea-pig: Ordinary ovaries containing very large, good follicles were found. The thyroid did not show hypertrophy.

2. Experiment BB (June 1936). Implantations were made every four days. First serial guinea-pig: There were larger than medium-sized, good follicles. In addition, luteinizing connective tissue atresia, well formed interstitial gland bodies and interstitial gland in medulla,

as well as lutein rings in follicles, which were at the stage of early connective tissue atresia, were found. The thyroid showed marked hypertrophy. Second serial guinea-pig: There were very large and also mature follicles. Besides pseudolutein bodies, some rudimentary interstitial gland bodies, large follicles with net granulosa, luteinizing connective tissue atresia and well developed interstitial gland in medulla were noted. The thyroid was hypertrophic, but the hypertrophy was less marked than in the first guinea-pig. Third serial guinea-pig: There were fully matured follicles as well as follicles with degeneration of the granulosa. Fairly well developed interstitial gland in medulla, interstitial gland bodies and luteinizing connective tissue atresia were found. The thyroid was without hypertrophy.

Group 3.—In this group anterior lobes of human pituitary glands were implanted serially into two female guinea-pigs with the same average weight as the guinea-pigs of the preceding groups. Two experiments were carried out which gave similar results. In both cases the second serial guinea-pig received each day, for four successive days, one implant of one fourth of a human anterior lobe after it had been removed from a first serial guinea-pig. Examination took place on the fifth day. In the first experiment fourths of a human gland were implanted into the first serial guinea-pig at the rate of one a day, and each fourth was allowed to remain for one day; it was then reimplanted into the second serial animal. In the second experiment all four pieces were implanted in the first animal at the same time and left for four days; they were then reimplanted into the second, one on each day. The results were, in both experiments, very similar. We shall cite the first experiment as an example.

First serial guinea-pig: The ovaries consisted largely of follicles undergoing luteinizing connective tissue atresia; large interstitial gland bodies and pseudocorpora lutea, good interstitial gland in the medulla and luteinization of theca interna around preserved follicles were found. The follicles, however, did not grow to full size. The thyroid was hypertrophied. Second serial guinea-pig: Mainly normal growing follicles were seen, but there were also large follicles with net granulosa, as well as maturing and many large, fully matured follicles. Definite but not marked interstitial gland was observed in the medulla. The thyroid did not show hypertrophy.

We may conclude, then, that with serial implantation of the human anterior lobe the results are similar to those obtained with implantation of the anterior lobe of beef, the intensity of the luteinizing processes being diminished and the maturation processes intensified in the second serial animal receiving the implanted human anterior lobe.

COMMENT

Altogether seventeen experiments in which anterior lobes of bovine and human pituitary glands were implanted serially into guinea-pigs were made. According to the mode of experimentation and the results obtained, these experiments can be divided into two classes, namely, a first class consisting of eleven experiments and a second class consisting of six experiments.

The first class comprises nine experiments with bovine and two experiments with human anterior lobes. These experiments were carried out during the winter, spring and early summer. In seven of the experiments with bovine and in the experiments with human anterior lobes, the pieces were implanted into the second and third serial guinea-pig at the rate of one a day; in the first guinea-pig they were either implanted at the rate of one a day, or all were implanted simultaneously on the first day. In only two of these experiments were the pieces of glands implanted at the same time in all the serial guinea-pigs. In the second class the pieces were implanted in all the serial guinea-pigs at the same time. Moreover, these experiments were carried out at the end of a very hot summer, at a time, therefore, when the resistance of the animals was probably relatively low; two of the guinea-pigs used in these experiments died prematurely, and some others lost weight during the experiments.

Both classes, considered together, indicate that the mode of implantation of the pieces helps to determine which hormone shall predominate. Daily implantation of one piece of the fresh bovine gland in the first serial guinea-pig induces mainly atresia-producing action together with very slight processes of luteinization, because in this case some of the pieces are left in the animals for only a short time and their store of atresin has not yet become exhausted at the time of examination. If the pieces of the fresh gland are implanted at the same time and allowed to remain for more than one day, the atresia-producing action diminishes and the processes of luteinization become more pronounced. If the pieces are implanted simultaneously in the first serial guinea-pig and allowed to remain as long as four days, even processes of maturation may begin to appear in some cases. There is a second factor which influences the results. If an animal loses weight following the implantation, or if the weight of the animal was low in the beginning of the experiment, the atresia-producing effect is fortified, because a state of undernourishment is unfavorable to the full development of the ovarian follicles and may accelerate the onset of follicular atresia.

The eleven experiments of the first class indicate that soon after implantation the substance which induces follicular atresia predominates. This is gradually weakened in its action, probably as the result of con-

tinued extraction of atresin from the implanted pieces of tissue, and then a time arrives when the luteinizing effects have a chance to assert themselves. After the substances responsible for luteinization have been sufficiently extracted, the pure maturation effects may become manifest. If, as is the case in the use of the anterior lobe of the human pituitary gland, luteinization together with premature maturation in smaller follicles predominates from the beginning, because of the lack of atresin, the substances that are responsible for the luteinization and the premature maturation may be at least partly eliminated in the course of serial implantations, presumably as the result of progressive extraction, and then again the maturation processes predominate.

The results of serial implantations agree thus very well with the effects observed in those experiments in which the pieces of the anterior lobe were allowed to remain for a longer time in the first serial animal; in this case we also noticed that the processes of luteinization began to predominate; a greater part of the atresia-producing substance would thus have been extracted or otherwise inactivated at the end of the first serial implantation.

The results obtained in the second class of experiments indicate that under certain conditions, affecting the weight or health of the animal unfavorably, atresia-producing action may continue to be effective throughout the serial implantations; the atresin remaining in the piece acts then in conjunction with a deficiency of available foodstuffs and thus leads to degenerative processes in the follicles. In other cases all the ovarian hormones may become inactivated or extracted during the second and third serial implantations, and the ovary returns, therefore, to its normal state uninfluenced by the implants of anterior lobe of the pituitary. In the course of the serial implantations the thyroid hormone also tends to be extracted and to lose in effectiveness; however, the degree to which this occurs varies under different conditions.

These experiments contribute data which make possible an analysis of the factors of significance in the destructive action of atresin from the anterior lobe of the pituitary gland on the ovarian follicles; furthermore, they contribute to the understanding of the extensive processes of luteinization, which likewise prevent normal development of the follicular apparatus.

SUMMARY AND CONCLUSIONS

Serial implantation of the anterior lobes of the pituitary glands of cattle in guinea-pigs differs somewhat in its results in accordance with the method of implantation used. If in the second and third guinea-pigs fourths of the anterior lobe of a pituitary gland are implanted at the rate of one a day for four consecutive days, atresia-producing action lessens

and various processes of luteinization become accentuated, and in some cases, in the end, maturation of the granulosa of large follicles predominates.

If also in the second and third serial guinea-pigs the pieces of the anterior lobe of a pituitary gland are all implanted at the same time, in some cases, likewise, the processes of maturation in large follicles begin to predominate. But in other cases marked luteinization with production of large follicles and, perhaps, even beginning maturation may occur already in the first serial animal; and in the second and third serial guinea-pigs the effect of the implanted anterior lobe on the ovary may be lost. In still other instances atresia-producing action continues to assert itself under these conditions.

After serial implantation of anterior lobes of human pituitary glands into guinea-pigs there is likewise relative loss of the luteinizing, and increase in the maturation, effects. The thyroid-stimulating hormone, as a rule, is diminished or lost in the large majority of these serial implantations.

We may then conclude that (1) in confirmation of some of our earlier observations, the mode of action of anterior lobes of the pituitary glands of cattle implanted into guinea-pigs varies to a certain extent in accordance with the mode of implantation, and that (2) after implantation of anterior lobes of bovine and human pituitary glands different effects are exerted by these glands in a definite sequence, which bears some relation to the changes which are produced in these glands by their treatment with certain chemical substances in vitro previous to implantation.

EXPERIMENTAL ENDOCARDITIS

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I. ENDOTHELIAL CHANGES DUE TO PRESSOR EPISODES

In the series of studies of which this is the first, the underlying thesis concerns the histologic alterations that are associated with the so-called pressor episode, i. e., the undue accentuation of smooth muscle contraction (whether in the vascular system, the sphincters, the uterus or another organ) that occurs with the ARS phase which has been discussed by Petersen.[1] This phase is associated with undue general or local anoxemia. The resulting functional changes are represented by various degrees of stimulation, fatigue or even death. The histologic evidences are presumably limited to the more localized effects of undue shortening of smooth muscle and vary from functional alterations to necrosis. Fundamentally the changes do not involve bacterial effects and inflammation in the usual sense; the regions affected may, however, form foci of localization for bacteria. The studies included the effect on the heart (endocarditis and myocarditis), the stomach (Nedzel[2]), the brain and other organs.

The establishment of a definite connection between endocarditis and bacteria led to attempts to explain this connection and to uncover the causes of localization of bacteria on the valves. The well known early experiments of Wysokowitsch[3] and Ribbert,[4] in which they produced gross trauma of the heart valves in animals and afterward introduced bacteria into the animals, were successful in producing endocarditis but did not answer the question as to the development of endocarditis under ordinary conditions. Neither have the observations of Lissauer[5] and Saltykow[6] thrown light on the pathogenesis of endocarditis, although these investigators produced experimental endocarditis without such gross trauma of the heart valves. Why the valves, especially the valves

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3. Wysokowitsch, W.: Virchows Arch. f. path. Anat. **103**:310, 1886.
4. Ribbert, H.: Fortschr. d. Med. **4**:1, 1886.
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6. Saltykow, S.: Virchows Arch. f. path. Anat. **209**:126, 1912.

of the left side of the heart, where the stream of blood is so vigorous and presents the most unfavorable mechanical condition for the quiet settlement of bacteria, should be selected by the micro-organisms, and not the endothelium of other parts of the heart, has not been explained. This led certain schools of bacteriologists to accept a theory of bacterial selectivity, though as early as 1887 Weichselbaum,[7] on the basis of his own and other authors' works, concluded that acute endocarditis is caused by bacteria of many varieties: (1) by those which cause other well known types of acute inflammation and (2) by those which one does not commonly associate with this kind of pathologic process, the pathologic or physiologic rôle of which in the body one does not know. Later observations did not support the theory of bacterial selectivity, so the more recent investigators have concentrated their attention on the host.

Hutyra and Marek [8] thought that the bacteria which circulate in the blood stream probably rarely localize directly on the surfaces of the valves but are arrested in the capillaries of the valves and also, at the same time, in the blood vessels of the heart muscle. The reason why the localization of bacteria of the second mentioned variety occurs on that surface of the valves which is turned toward the lumen of the heart lies, according to Eisenman (quoted from Hutyra and Marek [9]), in the peculiar arrangement of the blood vessels in the cardiac valves. The micro-organisms which have localized and their toxins cause an inflammatory condition in the valves and in the wall of the heart. The surfaces of the valves become rough, and masses of thrombi are deposited. Müller and Glass [9] stated that acute endocarditis can be produced experimentally by the introduction into the blood of various forms of bacteria, particularly if the valves have been previously affected with inflammatory processes. Leschke [10] concluded from Virchow's and Orth's works that the reason why endocarditis is found on the edges of the valves lies in the invasion of these edges by bacteria floating in the blood stream. This invasion is a result of a disposition on the part of the margins of the valves brought about mechanically by their impingement on each other.

Wadsworth [11] noted that horses immunized with killed pneumococci often acquire endocarditis. Birkhaug [12] succeeded in producing endo-

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10. Leschke, E., in Kraus, F., and Brugsch, J.: Specielle Pathologie und Therapie, Berlin, Urban & Schwarzenberg, 1919, vol. 2, p. 1072.

11. Wadsworth, A. B.: J. M. Research **39**:279, 1919.

12. Birkhaug, K. E.: J. Infect. Dis. **40**:549, 1927.

carditis in rabbits by a single intravenous injection of streptococci after establishing in each a subcutaneous focus of the same organisms. Freifeld [13] produced endocarditis in rabbits by injecting staphylococci, but only after the animals had been treated by repeated injections of killed streptococci. Silberberg [14] obtained the same results with staphylococci, but previously he had injected a solution of lithium carmine. Siegmund [15] produced endocarditis in rabbits by injecting daily increasing doses of colon bacilli and staphylococci. Semsroth and Koch [16] produced endocarditic lesions in two ways: (1) by preceding the injection of staphylococci by an intravenous injection of killed streptococci; (2) by preceding it by an intravenous injection of casein. Rinehart and Mettier [17] showed that infection of guinea-pigs maintained on an adequate diet usually produces no significant lesions in the heart valves. In uncomplicated scurvy definite atrophic and degenerative changes occur, but in scurvy with added infection striking lesions of a combined degenerative and proliferative character develop in the heart valves with considerable frequency. Siegmund [18] in his studies of the pathogenesis of endocarditis, especially its early manifestations, came to the conclusion that the basis for the development of thrombo-endocarditic processes should be looked for in the reaction of the indifferent mesenchymal tissue in the endothelial layer. Earlier Kuczynski and Wolff,[19] by repeated injections of cultures of Streptococcus viridans into mice, frequently found a subendothelial proliferation of plasma cells and compared it to the early manifestations of endocarditis in man. Jaffé [20] in a comparative study of numerous cases of septicemia in which there were no grossly visible endocardial changes concluded that at first the endothelial cells swell and become necrotic and that the bacteria settle down in the necrotic tissue. If the necrosis spreads further and the bacteria invade the necrotic tissue, the whole vegetation is gradually destroyed by suppuration, and an ulcer results. Jaffé also emphasized the fact that the micro-organisms are present chiefly on the surface of the thrombi (which are of secondary nature) and are not found in the deeper portions. Semsroth and Koch [21] noticed in their experimental endocarditis the superficial accumulation of cocci, as did many other

13. Freifeld, H.: Klin. Wchnschr. **7**:1645, 1928.
14. Silberberg, M.: Virchows Arch. f. path. Anat. **267**:483, 1928.
15. Siegmund, H.: Verhandl. d. deutsch. path. Gesellsch. **19**:114, 1923.
16. Semsroth, R., and Koch, R.: Arch. Path. **10**:61, 1930.
17. Rinehart, J., and Mettier, S.: Am. J. Path. **10**:61, 1934.
18. Siegmund, H.: Virchows Arch. f. path. Anat. **290**:3, 1933.
19. Kuczynski and Wolff: Verhandl. d. deutsch. path. Gesellsch. **18**:47, 1921.
20. Jaffé, R.: Proc. Inst. Med. Chicago **9**:318, 1933.
21. Semsroth, R., and Koch, R.: Arch. Path. **8**:821, 1929.

investigators. Ribbert [22] in discussing the rôle of mechanical trauma in the development of endocarditis came to the conclusion that it is of the utmost importance to have an endocardium previously injured by the toxins. After an injury of this type the bacteria settle on the valve.

Siegmund, Dietrich and Freifeld (quoted from Semsroth and Koch) thought that the bacteria localize on the endocardium because of an altered reaction of the endocardium as a result of a peculiar state of immunity. Semsroth and Koch,[21] on the basis of their observations, suggested that a decisive factor in the pathogenesis of endocarditis is a disturbance of the detoxifying ability of the reticuloendothelial system. In the presence of this disturbance, amounts of toxic bacterial products which are practically innocuous to the tissues of the normal animal lead to inflammatory lesions of the endocardium. This cellular damage in turn enables bacteria to localize on the endocardium. Eppinger [23] stated that dogs treated with allylformate present changes similar to the ones that have been proved to represent a toxic injury of the endothelium. This serous inflammation is antecedent to the endocarditis, and the bacteria at first invade the valves that have been injured in this manner.

Eppinger and his associates [24] presented experimental evidence indicating the importance of a change in the permeability of the valvular endothelium. Von Albertini [25] in describing the development of endocarditis spoke of primary changes in the endocardium—a surface endothelial lesion.

From this brief review, one sees that practically all investigators are coming to the conclusion that the bacteria, without any special selectivity, more or less passively localize on the endothelium of the valves, that some sort of injury to these valves (gross or microscopic, physicochemical or physiologic) is the predisposing factor and that the bacteria are localized from the passing blood stream.

The later investigators have also pointed out that there are certain changes in the endothelium during which it becomes more permeable and adhesive, a condition which facilitates the localization on, and permeation of, the membranes by bacteria (Burrows [26]). This has been shown on clinical material by Petersen.[1]

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22. Ribbert, H., in Henke, F., and Lubarsch, O.: Handbuch der speciellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1924, vol. 2, p. 204.

23. Eppinger, H.: Deutsche med. Wchnschr. **60**:1740, 1934.

24. Eppinger, H.; Kaunitz, H., and Popper, H.: Wien. klin. Wchnschr. **47**: 225 and 262, 1934.

25. von Albertini, A.: Schweiz. med. Wchnschr. **16**:200, 1935

26. Burrows, H.: Some Factors in the Localization of Disease in the Body. London, Baillière, Tindall & Cox, 1932.

One observes endocarditis usually in northern latitudes, and at certain seasons (late winter and spring) mostly among young persons. Petersen [1] presented the evidence that blood pressure constantly changes and that this change is meteorologically conditioned.

In following the functional status of endothelial permeability day by day, Petersen [1] found that the functional state varies. As the organism as a whole swings through its regular (or irregular) phase difference that is designated ARS (anabolism, reduction, spasm) to COD (catabolism, oxidation, dilatation) and vice versa, the endothelium becomes more permeable during the COD phase and less permeable with the ARS phase. The ARS phase involves a pressor increase, and the greater this is, the greater the effect of chemical stimulation in certain parts of the endothelium and the greater the possibility for anoxemic conditions in certain portions of the organism. With periods of anoxemic stimulation or following mechanical stimulation, the endothelium, as before stated, not only becomes more permeable but becomes sticky—a physicochemical change that involves changes in the electrical charge.

These changes of phase may be initiated by many general factors (endocrine, nutritive, toxic) but are most commonly associated with swings in biologic rhythm that are set in motion by the effort of the organism to adapt to the cyclonic shifts in the atmosphere.

There is every reason to believe that all the endothelium of the body will be more or less involved in such periods of relatively lessened or increased activity, of lessened or increased adhesiveness.

Incidental to this changing rhythm of ARS and COD, the pressor relations of the vascular system are altered. Periods of high blood pressure (ARS phase—spasm) are followed by periods of low blood pressure (COD phase); with a period of spasm, anoxemia prevails in certain tissues; this causes stimulation; products of incomplete tissue metabolism are released and thrown into the circulation; capillaries are dilated (carbon dioxide accumulates), and the diastolic blood pressure falls. The vascular bed becomes wider.

Consider what such a pressor episode means to the heart. The increase in pressure means more work for the left side of the heart; the right will not be affected to any great extent. The margins of the valves impinge on each other more forcibly, and as a consequence the marginal endothelium in the region of contact is mechanically stimulated to a greater degree. The marginal zones of the aortic and the mitral valve are selectively stimulated and selectively more adhesive.

There are, however, coincident changes in the vessels of the valves. One ordinarily disregards them in one's consideration, but injection of pitressin (betahypophamine), which produces a response in pressure, frequently is followed by a hemorrhage into the leaflets of the valves, and even ordinary pressor episodes are probably associated with con-

siderable nutritive change in the tissue of the valves. These considerations, based on extensive observations (Petersen [1]), explain the onset, the time of the onset and the localization of bacterial endocarditis.

The aforesaid observations bring out a thought, that vasomotor instability, with its greater pressor peaks, may have a direct influence on, if it is not the real background for, the development of endocarditis. If this is true, by inducing pressor episodes experimentally one should be able to produce bacterial endocarditis artificially.

As mentioned, the later investigators have pointed out that the endothelium of the heart valve is a primary seat for passing bacteria, so changes in the valvular endothelium should be looked for in the first place.

EXPERIMENTS

For the production of artificial pressor episodes in this study, use was made of intravenous injections of pitressin (betahypophamine).

The experiments were carried out in the following manner: Preferably young dogs, from 4 to 8 months old and from 8 to 12 pounds (3.5 to 5.5 Kg.) in weight, healthy and well fed, were used. Five of these each received a single injection of pitressin (1 cc.) intravenously. Dog 1 was put to death within an hour, dog 2 within three hours, dog 3 within seven hours, dog 4 within twenty-four hours, and dog 5 within forty-eight hours after the injection, by bleeding. Histologic slides were made of the valves (preferably the bicuspid), and the endothelium was examined.

Microscopic Observations.—The endothelial cytoplasm became swollen; the nuclei no longer projected above the surface and appeared thicker and rounded. In all this group of dogs the changes in the valvular endocardium were not well pronounced, so another group of three dogs was added, in which the technic was modified in such a manner that the pressor episodes would be more pronounced and more prolonged. This was accomplished by giving repeated injections of pitressin, i. e., four consecutive intravenous doses at two hour intervals. The doses were 0.5, 0.65, 0.75 and 1 cc. These dogs were disposed of (also by bleeding) after the last injection as follows: dog 6 within an hour, dog 7 within twenty-four hours and dog 8 within forty-eight hours. When the heart valves (mitral) were examined for endocardial changes, these were found well pronounced, especially in dogs 7 and 8.

The different stages of the changes will be presented, step by step, as they were observed. Each valve showed the endothelial changes at many different stages. Eight dogs treated with single injections and two dogs treated with repeated injections did not show changes and were disregarded.

The findings are now presented in a series of photomicrographs:

Figure 1 presents a photomicrograph of a normal mitral valve of a dog. One notes here the superficial position of the endothelium. The nuclei, flat and elongate are laid out horizontally; they slightly project above the surface. The cytoplasm of the endothelial cells is of relatively small amount, and the subendothelial structures are relatively firm and compact.

After the injections of pitressin one notes gradual changes in the endothelium of the valve (dog 6, fig. 2). One notes that some of the endothelial nuclei are a little shorter and thicker. Their relationship to the subendothelial layer changes with the thickening of the endothelial surface on account of thickening of the

cytoplasm. This goes further (dog 6, fig. 3); the cytoplasm of the endothelium definitely swells more, and the nuclei no longer project above the surface. The nuclei become thicker, and their ends are rounded.

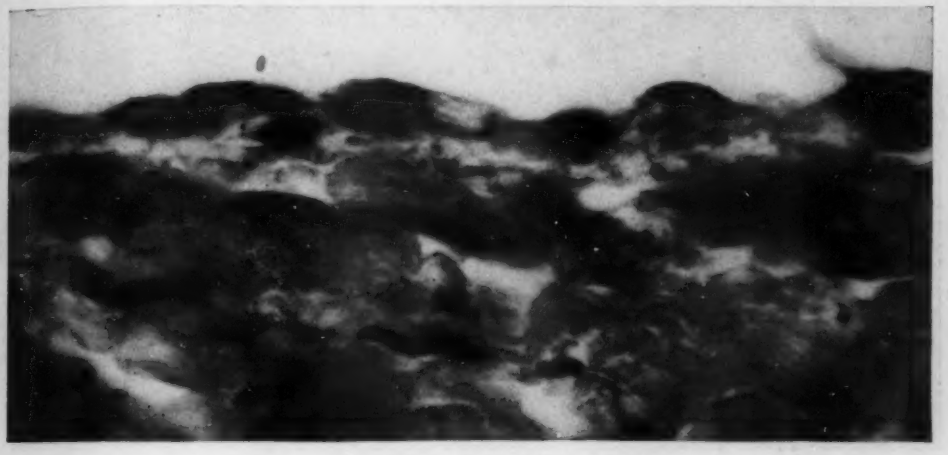

Fig. 1.—The endocardial surface of a normal mitral valve of a dog; × 1,500; oil immersion.

Fig. 2 (dog 6).—The initial change in the endocardial surface of the mitral valve after a pressor episode induced on March 2, 1936; × 1,500; oil immersion.

The swelling of cytoplasm continues beyond the endothelial nuclei (dog 6, fig. 4), and they no longer project from the valvular surface. The change of the endothelial cells involves the nuclei (dog 7, fig. 5), which become rounded and elongate, and their axes, usually parallel to the surface, change. There is also evidence of cell division.

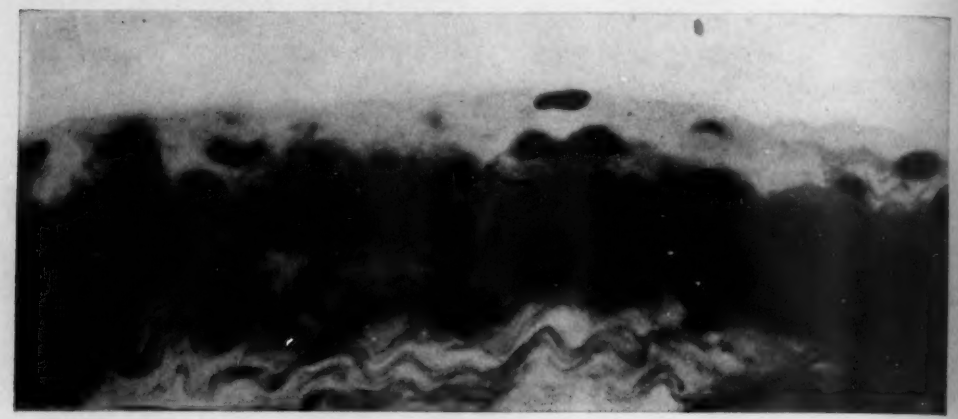

Fig. 3 (dog 6).—Swelling of the endothelial layer of the endocardial surface of the mitral valve after a pressor episode; × 1,500; oil immersion.

Fig. 4 (dog 6).—Swelling of the endothelial cytoplasm beyond the nuclei after a pressor episode; × 1,500; oil immersion.

Later the axes of the nuclei (dog 7, fig. 6) become wholly vertical to the valvular surface, which is still intact. In figure 7 (dog 7) the nuclei become more separated from the subendothelial layer. Their axes have all changed position from one parallel to the surface to one vertical to it. The smooth valvular sur-

face now also changes; it becomes roughened, interrupted. Some of the endothelial cells appear to show vacuolation, and their attachment to the subendothelial layer appears less firm. These vacuolated cells begin to project freely on the

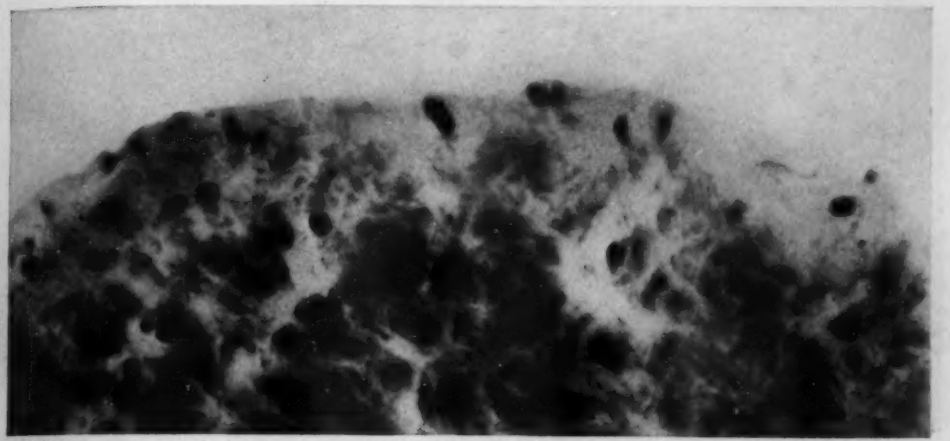

Fig. 5 (dog 7).—Endothelium after a pressor episode induced on March 3, 1936; × 1,500; oil immersion. Note the gradual change in length and position of the axes of the endothelial cells, also the evidence of cell division.

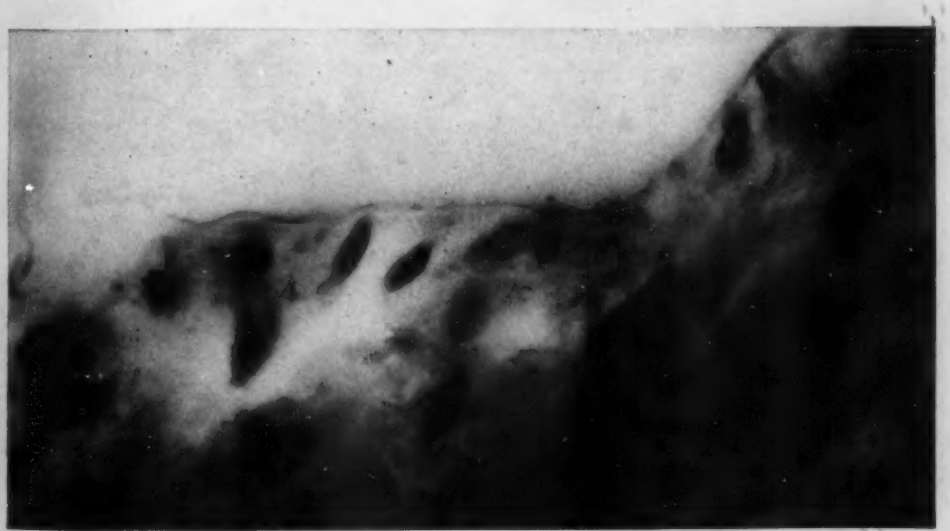

Fig. 6 (dog 7).—Endothelium after a pressor episode; × 1,500; oil immersion. The superficial endothelial cells are now placed vertically. The surface membrane is still intact.

surface (dog 7, fig. 8), being loosely attached to the subendothelial layer. The subendothelial cells are edematous. This hydration (stimulation) of the endothelial cells goes further, as demonstrated in figure 9 (dog 7). Enlarged cells are shown

at *A* and *B*. At *A* one may see swollen cytoplasm with a relatively intact nucleus; at *B*, a diffuse type of protoplasmic mass, replacing the cytoplasmic structure and presenting a vastly increased adhesive surface, as well as apparent nuclear dis-

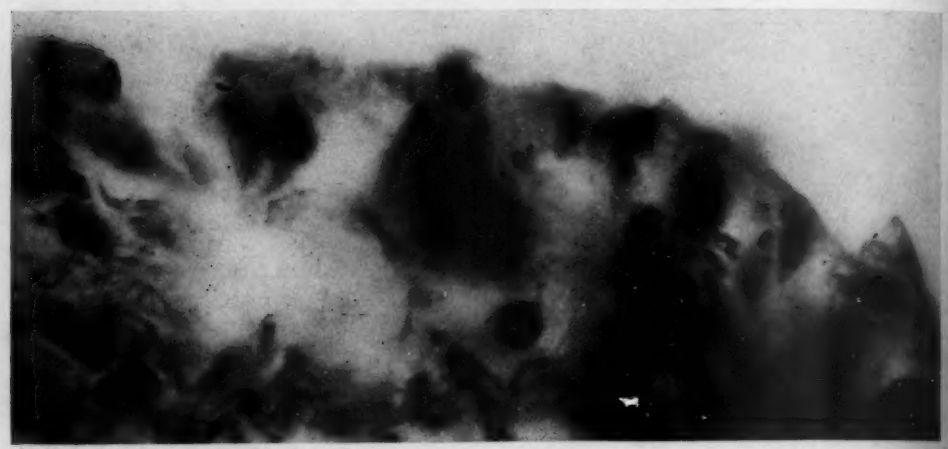

Fig. 7 (dog 7).—Endothelium after a pressor episode; × 1,500; oil immersion. The superficial membrane is roughened.

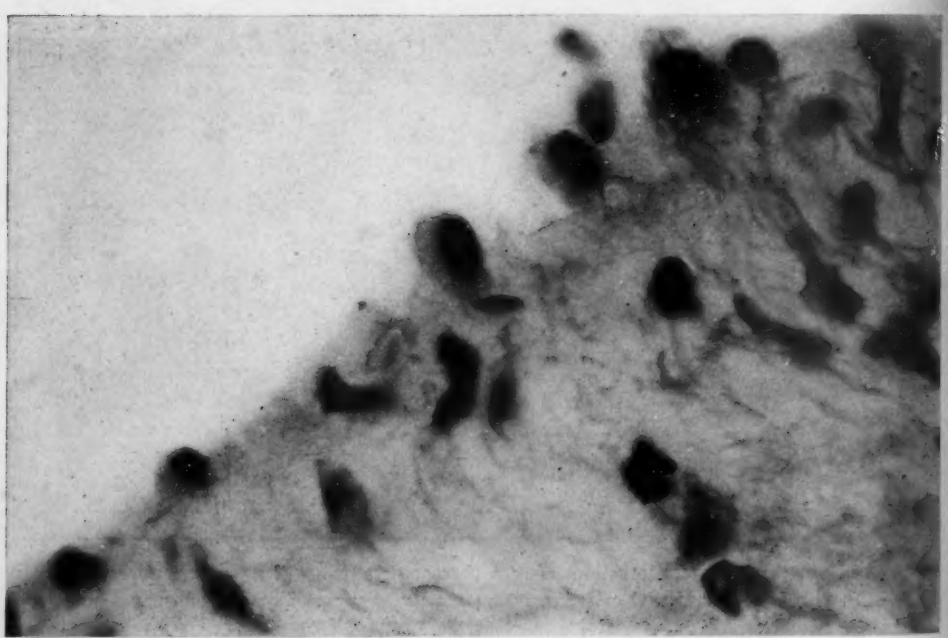

Fig. 8 (dog 7).—Endothelial surface after a pressor episode; × 1,500; oil immersion. The endothelial cells are loosely attached and vacuolated; the subendothelial structures are edematous.

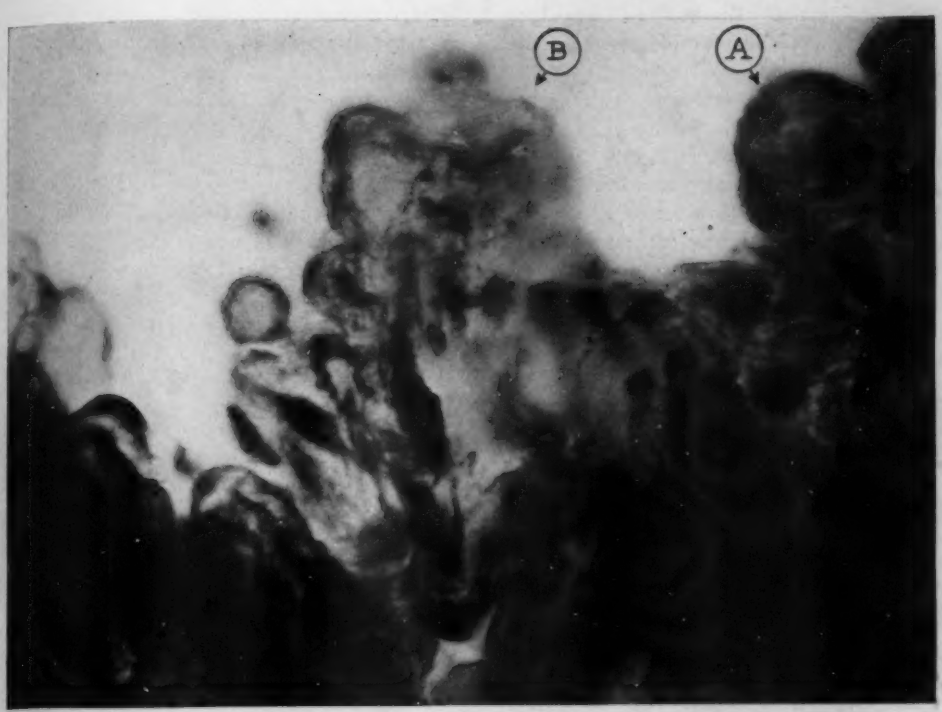

Fig. 9 (dog 7).—Endothelial cells visibly hydrated and stimulated after a pressor episode; × 1,500; oil immersion. *A* indicates enlarged cytoplasm with a relatively intact nucleus; *B*, a diffuse protoplasmic mass.

Fig. 10 (dog 7).—A proliferating endothelial nodule on the valvular surface after a pressor episode; × 1,500; oil immersion.

integration. One may find also (dog 7, fig. 10) distinct evidence of proliferation of endothelium in the form of clumps of apparently new-formed cells on the valvular surface.

In further stages of the changes on the valvular surface, one notes that the membrane on the valvular surface tends to present a stringy mucoid surface. That is plainly shown in figures 11 (dog 7) and 12 (dog 8). In figure 12, *A* and *B* present the same area but have been photographed in different focal planes. In one plane is seen the nuclear surface with the shadowy outlines of the cytoplasmic strands. The same area in another plane presents a definite picture of the stringy character of the cytoplasmic strands, which extend down into the layer of the endothelium. The photomicrograph also proves that here one is not dealing with platelet adhesions or thrombus formation but solely with endothelial structures that have been profoundly modified.

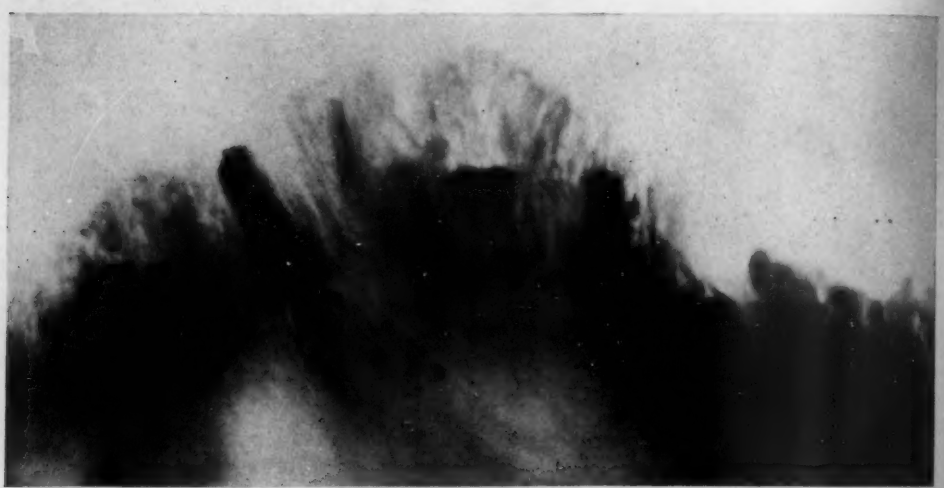

Fig. 11 (dog 7).—The endothelial surface after a pressor episode—spongy and adhesive; × 1,500; oil immersion.

At last one may select individual cells which will permit one to study the particulars of the transformation of the endothelial cells.

Figure 13 (dog 8) presents such an unusual protoplasmic mass, which apparently represents, modified to a great extent, an endothelial cell. It consists of widely flung protoplasmic masses that extend far into the blood stream. These gelatinous filamentous pseudopodia of the endothelial cytoplasm undoubtedly offer most convenient surfaces for the localization of bacteria from the blood stream, and their intimate connection with the structure of the valve is well shown.

Occasionally one may actually find bacterial inclusions in the heart valves of dogs in which pressor episodes have been induced with pitressin. In figure 14, arrows point to such inclusions in a heart valve of dog 8. The bacteria were not introduced subsequently to the injections of pitressin and must have been of endogenous origin. This proves that the endothelial surfaces in the dogs which underwent pressor episodes became suitable and favorable for localization of adventitious bacteria.

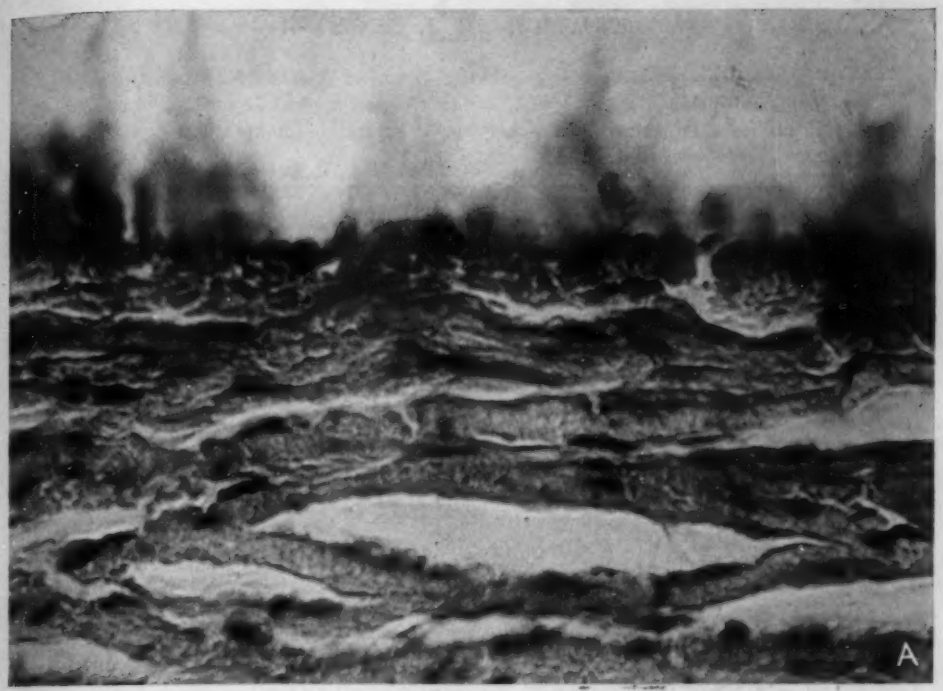

Fig. 12 (dog 8).—*A*, the endothelial surface after a pressor episode induced on March 4, 1936; × 1,500; oil immersion. A nuclear surface is shown, with shadowy outlines of cytoplasmic strands. *B*, the area shown in *A* but photographed in a different focal plane. Stringy cytoplasmic strands extend into the layer of endothelium; × 1,500; oil immersion.

Now the process may go in either of two ways: Bacteria may localize, penetrate and multiply in the valve, and one may expect typical bacterial endocarditis. Or, if no infection occurs, there may be organization of transient scars, with

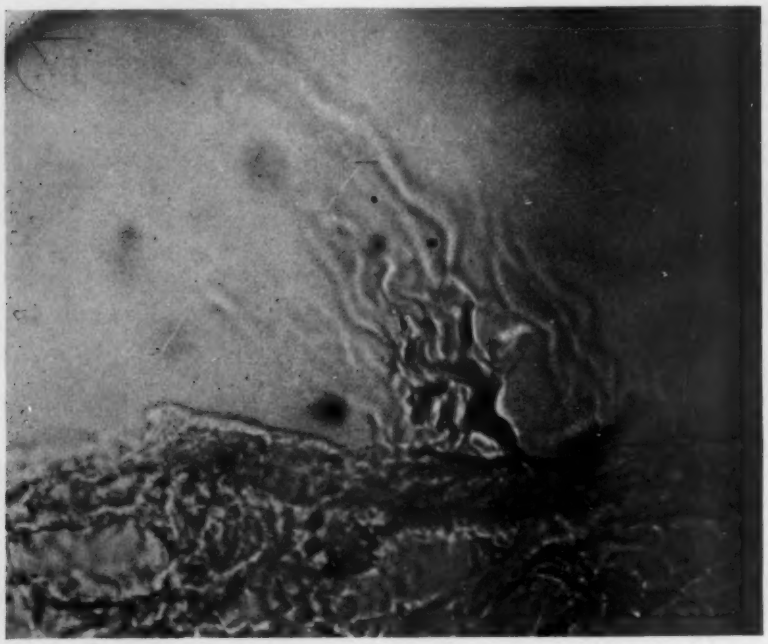

Fig. 13 (dog 8).—Endothelium after a pressor episode; × 1,500; oil immersion. Long protoplasmic streamers of a degenerated endothelial cell extend out into the blood stream.

Fig. 14 (dog 8).—Beginning of bacterial adhesion and phagocytosis by endothelial cells (arrows pointing). There had been no bacterial injection!

proliferation of connective tissue. Both outcomes of the effects of the pressor episodes on the heart valves have been observed and are described in later paragraphs.

Here I have presented the initial endothelial changes in the heart valve caused by pressor episodes induced by injections of pitressin. These changes are of such type that the endothelium becomes sticky and favorable for localization of bacteria from the blood stream. This will be discussed later.

The changes of endothelium due to pressor episodes are shown, step by step, beginning from an approximately normal state of the endothelial cell and progressing to a degenerated condition of the cell, with long protoplasmic streamers extending far out into the blood stream. The intact surface of the valve membrane changes into a roughened one; the endothelial cytoplasm becomes spongy and adhesive. This endothelial surface appears mucoid, stringy. Finally, one also occasionally observes localized micro-organisms of endogenous origin.

The endothelial changes due to stimulation of the cells almost always are seen in the endothelium of the valves and not in the endothelium of other regions of the heart because the margins of the valves impinge on each other rather forcibly and their endothelium is stimulated to a much greater extent than that of the other parts of the heart.

This also explains the predominance of the lesions on the valves of the left side of the heart in comparison with those of the right side. The valves of the latter side lead a relatively tranquil existence, while every emotional upset, every meteorological turn, indeed, every unusual vasomotor disturbance finds prompt reflection in a change of pressor levels of the greater circulation.

In the valves, especially those of the left side of the heart, one has conditions which provoke greater chemical stimulation, with greater possibility for anoxemic conditions, increased permeability and stickiness of the endothelium. The valvular endothelium, especially that of the mitral valve, being always more stimulated owing to its anatomic position and physiologic function, more readily responds to the stimulation in pressor episodes (invoked by injections of pitressin in the cases cited).

The endothelial changes described were observed in dogs destroyed not later than forty-eight hours after the induction of the pressor episodes. The animals to the last appeared normal and healthy except that in some of them reactions in the form of diarrhea were observed immediately after the injections of pitressin. These reactions did not last more than one or two hours, and after them the animals appeared perfectly normal and healthy, with the usual appetite.

SUMMARY

Artificially induced pressor episodes (obtained with pitressin) in dogs cause mechanical stimulation of the endothelial cells of the cardiac valves, particularly those at the contact surface of the mitral valve.

The expression of this functional stimulation may vary from evanescent alteration of the endothelial cells through stages of profound reaction, fatigue and death; histologically, these changes can be followed in microscopic sections, the alterations being demonstrable within an hour after the injection of pitressin.

The nuclei change their form and position; they undergo hydration and finally disintegrate; the cytoplasm swells and becomes vacuolated. The endothelial surfaces become mucoid and stringy. Some of the endothelial cells degenerate in such a way that long protoplasmic streamers may extend far into the blood stream. Cell surfaces so altered present an ideal condition for localization of bacteria present in the blood stream. Micro-organisms of endogenous origin may be observed localizing on endothelium.

Occasionally patches of proliferating endothelial cells can be observed on the surface of the valves.

While an artificial pressor episode has been dealt with here, it is obvious that the spontaneously induced condition can act in conditioning the endothelial surface by bringing about greater adhesiveness. The pressor episode thus becomes of significance for the initiation of true endocarditis.

II. BACTERIAL ENDOCARDITIS

In the preceding chapter I reviewed the literature and pointed out early recognition of the importance of injury to the heart as a conditioning factor in the development of bacterial endocarditis. Inasmuch as such gross lesions to the heart valve (Orth and Wysokowitsch [8]) are of no practical significance in the etiology, later investigators assumed the presence of lesions not microscopically detectable as of significance (including the allergic state).

Recent data on the variability of blood pressure in man in connection with adjustment to environment and on the effect of such alterations on metabolism in different tissues (Petersen [1]) led to investigation of the relationship between pressor episodes and possible changes in the valvular endothelium. The microscopic observations described here confirm a theoretical assumption of undue stimulation of endothelial surfaces with a pressor episode.

On microscopic examination it was found that pressor episodes stimulate the valvular endothelium and that with this stimulation the endothelium undergoes changes which are detectable and recordable. The endothelium becomes adhesive, and occasionally one sees implantation of wandering micro-organisms of endogenous origin.

From Petersen's observations in man it is known that in late winter and spring the endothelium in general is more permeable (stimulated) whereas in the summer and autumn the endothelium is less permeable. For the present problem this means that, other conditions being equal, the chance for bacterial adhesion is in general increased in the late winter and spring and that during the summer and autumn the possibilities for adhesion are lessened.

If it is true that the endothelium becomes adhesive (sticky) and that the implantation of endogenous bacteria from the blood stream is not a matter of chance, then the greater frequency of invasion of the blood stream and simultaneous change in the function of tissues receiving the bacteria, and not specific selectivity on the part of the invading organism, is of importance. The micro-organism may be relegated to the background and the conditioning factors that may influence its localization emphasized.

One should be able to prove experimentally (1) that after pressor episodes particulate matter and perhaps certain dyes adhere to the valves, (2) that bacteria injected into the blood stream also adhere and (3) that an ulcerative lesion may be produced with the gross and microscopic appearance characteristic of the endocarditis that one sees clinically in the human being.

Here I present experimental evidence in proof of the assumptions and demonstrate the close correlation between the pressor episode and localization of particulate matter and bacteria on the heart valve and, of course, primarily on its endothelial layer. When bacteria are so established, one may observe the development of true bacterial endocarditis.

EXPERIMENTS

The experiments were carried out on dogs. The dose of pitressin most useful for the purpose was 5 pressor units per kilogram of the weight of the dog. The intravenous injection of pitressin was followed in fifteen minutes by an injection (also intravenous) of a suspension of bacteria or of india ink. The bacteria used were Staphylococcus aureus and Streptococcus haemolyticus. The amount of bacteria given was, for the staphylococcus, the washing of a half of an agar slant and, for the streptococcus, the washing of a blood agar plate, both in 10 cc. of isotonic sodium chloride solution. In the experiment with india ink, the ink was given in a dose of 20 cc. of a 5 per cent solution in distilled water. After certain lengths of time (from one to twenty-three days) the dogs were killed by intravenous injection of ether, and the heart was removed. In cases in which bacteria were used, pieces of valves were obtained under sterile conditions and placed in suitable mediums. Pieces of valves were also embedded in paraffin and sectioned and the sections stained, first, slightly by hematoxylin and then by the Gram method and with eosin. If the bacteriologic finding was negative, the Gram-stained sections were not examined. For the detection of india ink, the sections of valve were slightly stained with hematoxylin and eosin. Adjacent sections of all of these sections were also stained by the ordinary hematoxylin and eosin stain for histologic studies of the valve tissue.

Of the eighteen experiments in which pitressin and Staph. aureus were used, the bacteriologic findings were positive in thirteen. Of the thirteen dogs, six died and seven were killed. On examination of sections for bacteria, whenever they were found they were observed mostly on the surface, either in the lesion, if the ulcer had already developed, or, at earlier stages, on the endothelium and closely adherent to it.

In six experiments in which pitressin and streptococci were used, there were two positive and four negative results. The cocci were seen on the endothelium.

Of four experiments with pitressin and india ink, two resulted in adherence of the dye. There were no deaths in these two groups.

For controls, two dogs were each given an injection of the staphylococcic suspension and four dogs were given only pitressin. In the first group the valves proved to be sterile, and in the second, no cocci were found.

Microscopic Observations.—The microscopic studies gave proof of the adhesiveness of the endothelium due to a pressor episode induced in a dog by injection of pitressin. Figure 15 presents the mitral valve of dog 9, which was killed within four days after the injection of pitressin and india ink. A carbon particle (shown by an arrow) has attached itself to the endothelial area of the mitral valve. Figure 16 shows the adhesion of a staphylococcus (indicated by an arrow) to the valve of dog 10, which was killed two days after the injection of pitressin and bacteria. In figure 17 one sees a picture of the same type, but the number of staphylococci is larger. The latter are all on the endothelial surface, showing the first stage of localization of bacteria on the live cells. This valve is from dog 11, killed within four days after a pressor episode produced by pitressin and followed by an injection of bacteria. Dog 12 died on the twenty-third day after the injection of pitressin and bacteria. Figure 18 shows the adhesion of bacteria to the seminecrotic endothelial surface of the mitral valve. With the adhesion one also sees bacterial penetration. Figures 19 and 20 present two slides of the mitral valves of dogs 13 and 14, which were destroyed on the twenty-third day after the injection of pitressin and staphylococci. The cocci adhere to the surface of the valve, but they also are multiplying in great numbers and are gradually and persistently penetrating through the seminecrotic tissue into deeper parts of the valve.

Macroscopic Observations.—The microscopic pictures described are accompanied by macroscopic changes in the valves. At first (within from twelve to thirty-six hours after the injection of pitressin and bacteria) one finds on the valvular surface slightly elevated round hyperemic lesions of a size varying from that of a pinpoint to that of the head of a pin. The lesions may be multiple and confluent. In some cases the involved site is pale pink or slightly reddish with a yellowish tint.

Figure 21 illustrates part of the mitral valve of dog 15, which was destroyed four days after the injection of pitressin and bacteria. Here one sees early lesions, consisting of a series of slightly raised reddish excrescences along areas of contact on the valvular surface. The outer portions of the lesions are indicated by two arrows. Some other small nodules were observed on the surface but are not shown in this photograph.

Figure 22 is taken from the mitral valve of dog 16, killed within seven days after the injection of pitressin and bacteria. Here also are shown early lesions (as indicated by arrows) extending along the valvular surface near the base of the valve. They appear as excrescences, slightly raised and of an orange color.

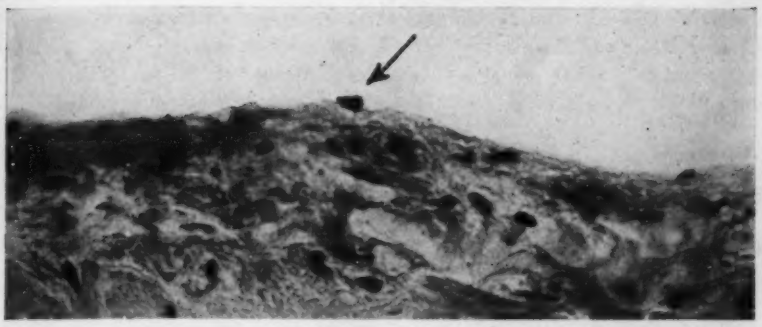

Fig. 15 (dog 9).—Adhesion of a carbon particle on the endothelial surface four days after the injection of pitressin and india ink on Jan. 26, 1934; × 900; oil immersion.

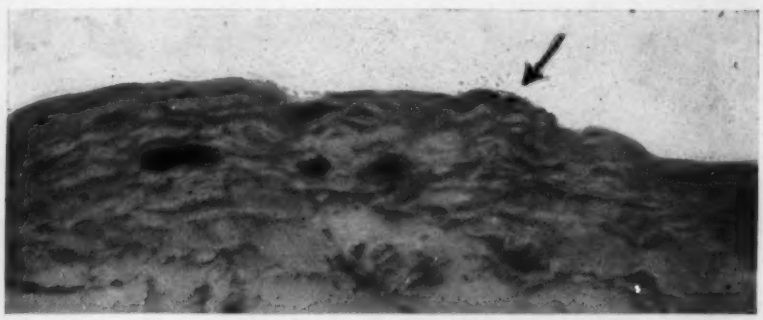

Fig. 16 (dog 10).—Adhesion of a staphylococcus to the endothelial surface two days after the injection of pitressin and a suspension of staphylococci on Jan. 25, 1934; × 900; oil immersion.

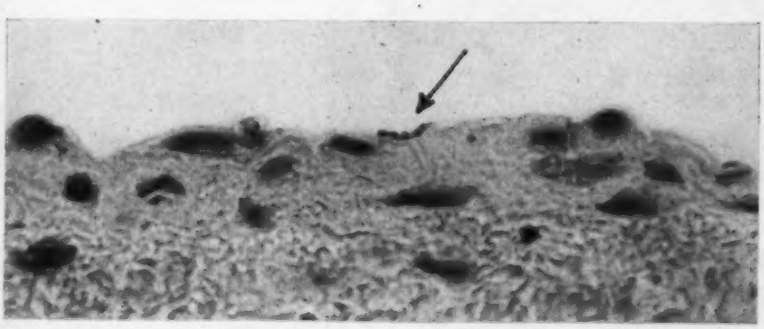

Fig. 17 (dog 11).—Adhesion of staphylococci to the endothelial surface four days after the injection of pitressin and a suspension of staphylococci on Jan. 26, 1934; × 900; oil immersion.

Dog 17, killed within twenty-four hours after the injections, shows on the mitral valve (fig. 23) clearly defined lesions, appearing as two cherry-red nodules projecting above the valvular surface (as indicated by the arrows on the right). The arrows on the left point to an additional number of flat hemorrhagic areas. On the only tricuspid valve showing lesions, obtained from dog 18, which was

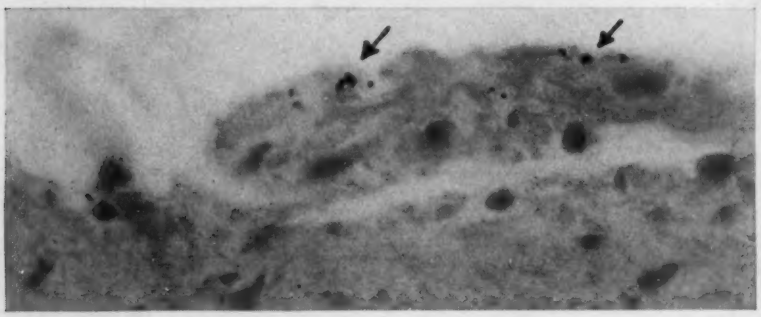

Fig. 18 (dog 12).—Adhesion of staphylococci on seminecrotic endothelial surface, with penetration into the deeper tissue twenty-three days after the injection of pitressin and a suspension of staphylococci on Dec. 21, 1933; × 900; oil immersion.

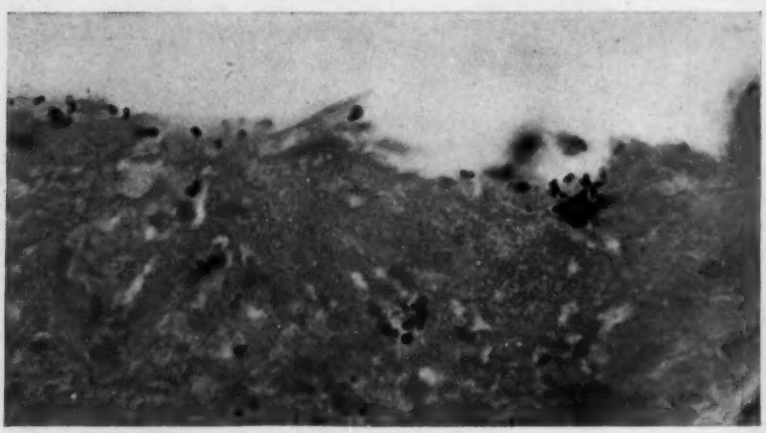

Fig. 19 (dog 13).—Adhesion of staphylococci on a seminecrotic endothelial surface, with multiplication and deep penetration of the micro-organisms into the valve twenty-three days after injection of pitressin and a suspension of staphylococci; × 900; oil immersion.

killed two days after the injections (fig. 24), one sees a characteristically edematous nodule at the middle (lower arrow) and a roughened hemorrhagic marginal zone extending along the base of the valve (the three upper arrows).

Figure 25 presents the mitral valve of dog 19, which died within three days after the injections. The lesions are very like those shown in figure 24. The

left arrow points to a definitely hemorrhagic lesion with a much roughened surface. The middle arrow indicates a typical button-like excrescence, and the right, a widely spread hemorrhagic zone. Figure 26 is taken from a mitral

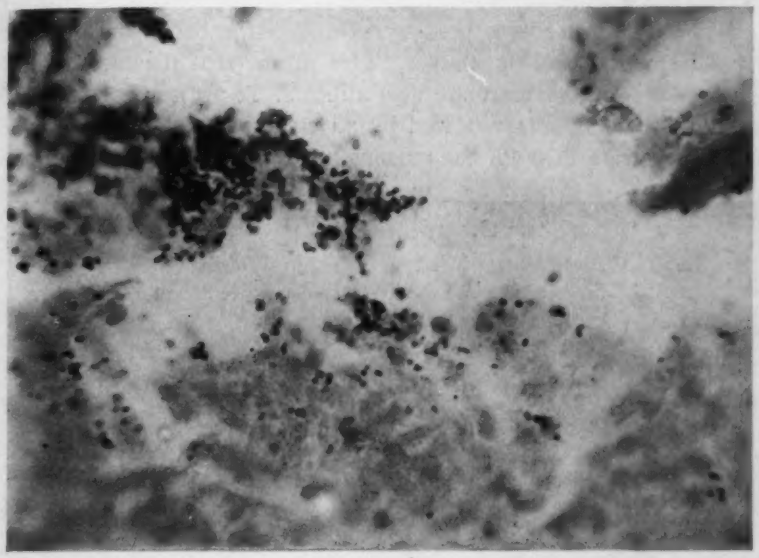

Fig. 20 (dog 14).—A greater number of micro-organisms twenty-three days after the injection of pitressin and a suspension of staphylococci on Nov. 6, 1933; × 900; oil immersion.

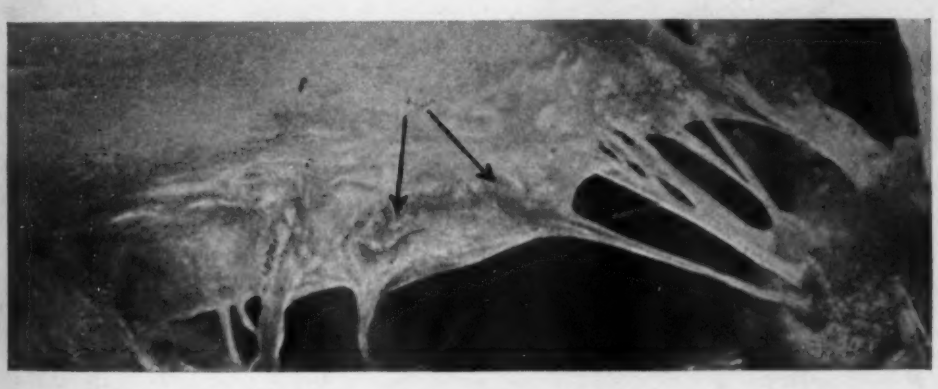

Fig. 21 (dog 15).—Gross appearance of early lesions on the surface of the valve four days after the injection of pitressin and a suspension of staphylococci on Feb. 26, 1934; × 6.

valve of dog 20, which died from the infection seventeen days after the injection of pitressin and staphylococci. The right arrow points to a large lesion with a hemorrhagic base and with innumerable small vegetations that project above the surface. In gross appearance the lesion obviously approaches that which is

observed in man. Some other characteristic lesions are indicated by the arrow at the left. The incision at the right is an artefact due to removal of tissues for microscopic study.

SPONTANEOUS BACTERIAL ENDOCARDITIS

In the occurrence of a pressor episode in the body, one deals with an involvement of all the tissues. This means that the membranes of the upper respiratory tract are also affected. A pressor episode (ARS phase of Petersen [1]) is followed by an enhanced opportunity for bacteria to penetrate through the mucous membranes; i. e., regional anox-

Fig. 22 (dog 16).—Gross appearance of early lesions of the valve near the base seven days after the injection of pitressin and a suspension of staphylococci on Jan. 13, 1934; × 6.

emia in the tissues of the mucous membranes will be followed by stimulation, increased permeability and greater freedom for the passage of bacteria. Clinically one can demonstrate that the pressor episode is the antecedent of the "cold" and the streptococcic sore throat (Petersen [1]).

Two factors in the constellation are then prepared by the pressor phase: (1) an enhanced opportunity for invasion of the blood stream from the mucous membranes (predominance of certain types of bacteria in the endocardial lesions) and (2) a nidus for localization.

If this is true, one may also be able to produce true bacterial endocarditis by merely inducing a pressor episode, without injection of bacteria into the blood stream.

Figure 27 presents a mitral valve of dog 21. The animal had injections of pitressin in doses of 0.5, 0.65, 0.75 and 1 cc. at two hour intervals. Seven days later the same procedure was repeated, and on the fifteenth day after the first series of injections the dog was found dead in its cage. No bacteria were injected into this dog, so the micro-

Fig. 23 (dog 17).—Arrows at the right indicate characteristic lesions; arrows at the left, hemorrhagic areas. These observations were made one day after the injection of pitressin and a suspension of staphylococci on Jan. 28, 1934; × 6.

organism which caused marked ulcerative endocarditis (shown in the photograph) apparently was of endogenous origin. The organism was Staph. aureus.

DETAILED STUDY OF EXPERIMENTAL BACTERIAL ENDOCARDITIS IN A DOG

Here I present a detailed description of ulcerative endocarditis as it developed in dog 22, which died seventeen days after the injection of

1 cc. of pitressin followed by a suspension of a culture of Staph. aureus.

A low power photomicrograph (fig. 28) of the mitral valve reveals infection and ulceration on both surfaces. At *A* great masses of bacteria in the form of clumped colonies (black stained masses) are infiltrating the connective tissue at parts of the valve. In addition to the solid masses, bacteria appear individually as innumerable cocci invading the interstitial connective tissue. There is much edema, and the structures in the immediate vicinity are wholly necrotic. At *B*

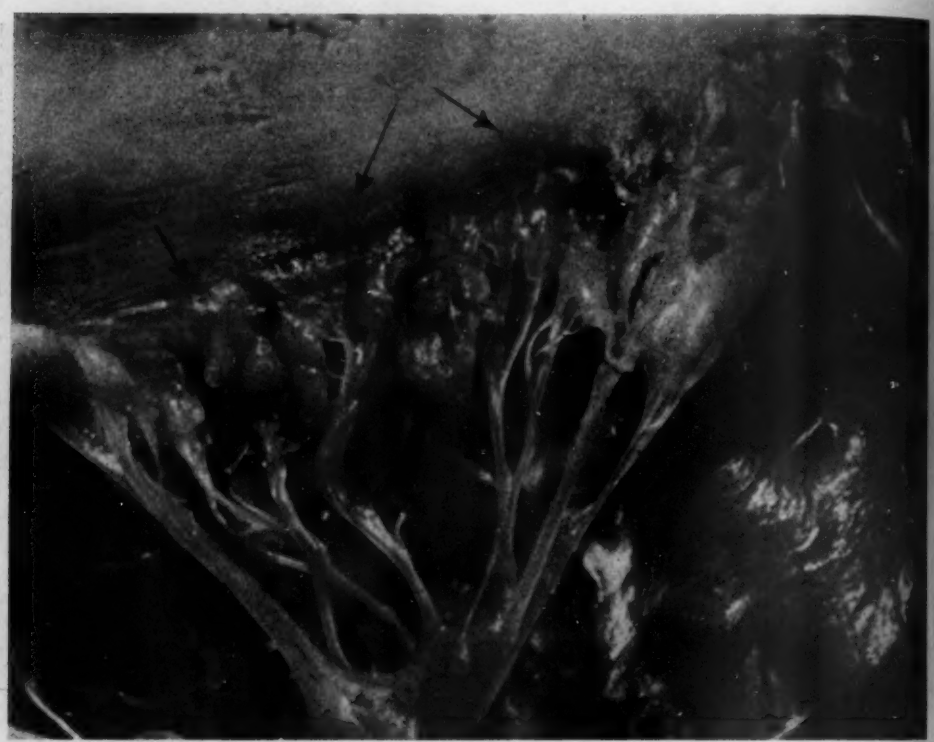

Fig. 24 (dog 18).—Tricuspid valve two days after injection of pitressin and a suspension of staphylococci; × 6. A series of hemorrhagic nodules is indicated along the base of the valve (upper arrows), a characteristically edematous nodule at the middle (right lower arrow) and lateral extension of the lesion (left lower arrow). The injections were made on Feb. 23, 1934.

one sees the terminal strand of a necrotic shred of endothelial infiltration of bacterial masses (stained black), with ulcerative necrosis of the subendothelial tissue as seen at *C*. The process is here very active but becomes less pronounced in the direction of point *D*. The bacterial infiltration here is considerably less. At *D* one sees also disintegrating endothelium, but the subendothelial tissues are reacting moderately.

The region from *C* to *E* is infiltrated. There are marked edema and cellular exudation. The extension of this led to a separation of the valve into two plates, from point *E* up to the free margin of the valve.

Figure 29 is a diagrammatic sketch of the same valve. The numbered rectangles represent the different portions of the valve which will be illustrated here by a series of photomicrographs (figs. 30 to 36).

Figure 30 presents zone 1 of the diagram. Here great masses of bacteria have completely filled the endothelial cells, forming a mosaic of deep staining clumps, which extend along the endocardial and subendocardial surface. At this part of the valve the clumps extend down into the subendocardium quite deeply, and individual cocci can be readily observed along the tissue spaces at *X* and *Y*.

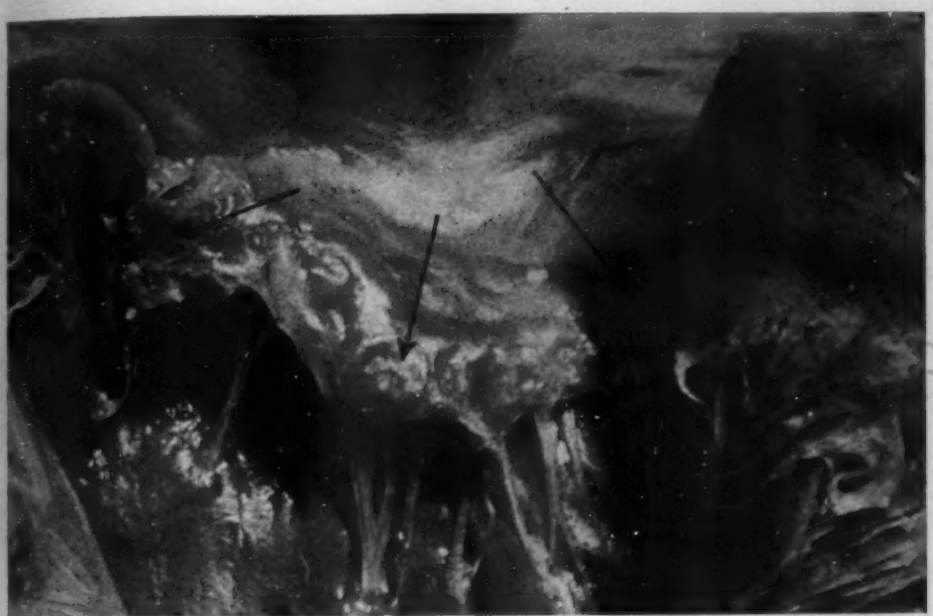

Fig. 25 (dog 19).—Characteristic vegetations on the valve three days after injection of pitressin and a suspension of staphylococci; × 6. The lesion indicated by the arrow at the left is markedly hemorrhagic. The arrow at the middle points to a characteristically edematous nodule. The arrow at the right points to a deep-seated hemorrhagic zone. The injections were made on Jan. 27, 1934.

On close inspection, organisms may be seen throughout the field. The cellular exudation is in part still mononuclear, but many polymorphonuclear leukocytes have appeared.

Figure 31 (zone 2) shows the protoplasmic masses already filled with bacteria. One may see as well the growth of organisms in the seminecrotic protoplasmic masses, as at *X*. The reaction of the subendocardial tissues here is yet limited, though the invasion of organisms can be observed to be proceeding.

Figure 32 (zone 3) shows the endothelial margin, where one can observe the bacterial invasion of the degenerating endothelial cells. In figure 33 (zone 4)

is seen a denuded surface with only a single endothelial cell, which is apparently still attached and filled with cocci.

Figure 34 (zone 5) presents the opposite valvular surface. Here one observes simply a purulent exudate in partly or wholly necrotic subendothelial tissue, cell débris, monocytes and polymorphonuclear leukocytes, the latter predominating. Bacteria are present everywhere. Tissue spaces (lymphatic) are everywhere dilated as seen in the left lower field. Figure 35 (zone 6) reveals a blood vessel underneath the endocardial surface, which here is little disturbed. The vessel is filled with red blood cells in considerable numbers, some monocytes and poly-

Fig. 26 (dog 20).—A characteristic lesion of the vegetative type in a dog seventeen days after injection of pitressin and a suspension of staphylococci; × 6. Hemorrhagic base leaflets (see arrows), markedly edematous, are shown. The injections were made on Dec. 21, 1933.

morphonuclear leukocytes. In that portion of the tissue adjacent to the longitudinal split in the valve, a polymorphonuclear exudate, with large numbers of organisms present, can be observed.

Finally, in figure 36 (zone 7) one observes bacterial growth in those parts of the valvular base where the tissue is already necrotic. The portion illustrated is that immediately beneath the great bacterial clumps that are evident in the margin of the photomicrograph.

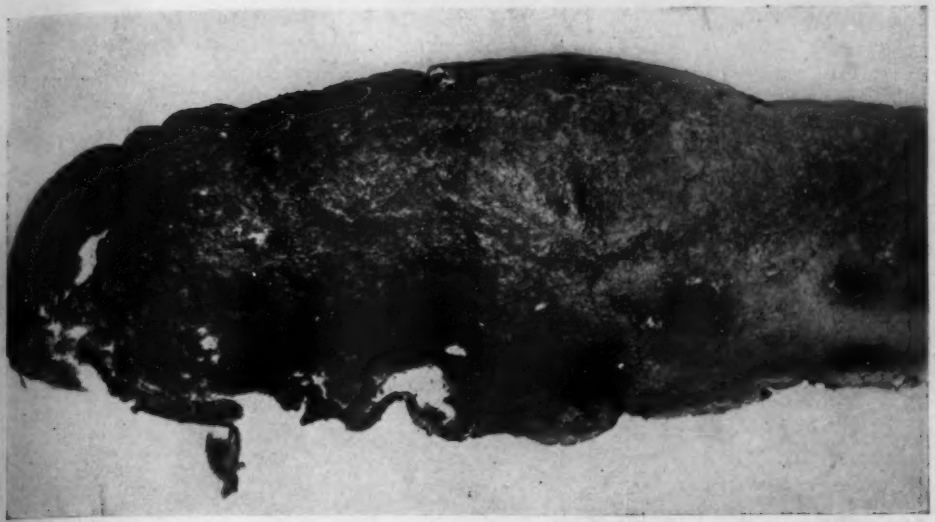

Fig. 27 (dog 21).—Mitral valve showing bacterial endocarditis after repeated injections of pitressin on March 3 and 26, 1936; × 100. The dog died on April 2. No bacteria were injected!

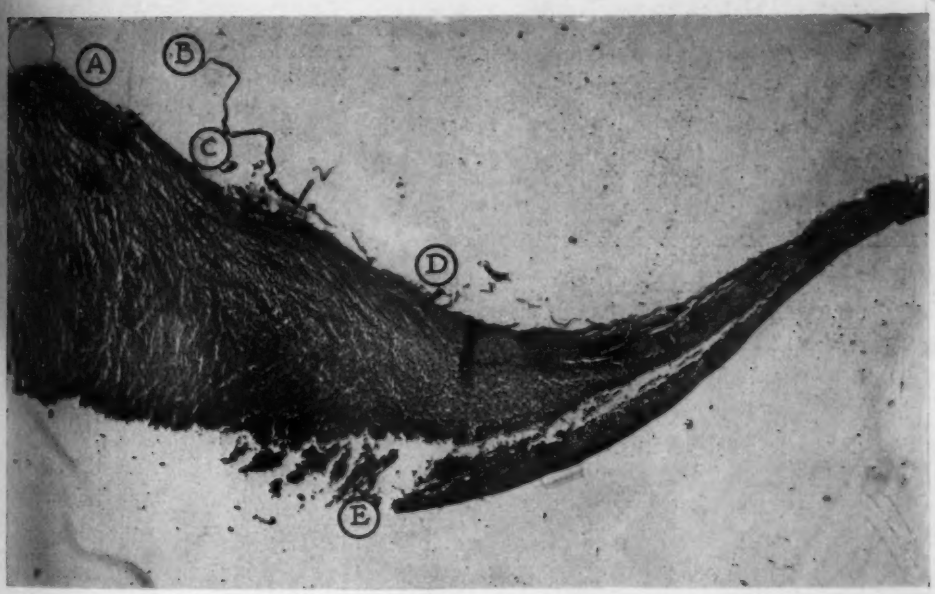

Fig. 28 (dog 22).—Mitral valve showing experimentally produced bacterial endocarditis seventeen days after the injection of pitressin and a suspension of staphylococci on Dec. 23, 1933. See text.

Swift [27] came to the conclusion that the micro-organisms in bacterial endocarditis are implanted in or on an abnormal valve. The bacterial endocarditis is thus of secondary origin in the vast majority of cases. Von Glahn and Pappenheimer [28] in their studies of the relationship between rheumatic and subacute bacterial endocarditis expressed the view that localization of bacteria occurs on the heart valve and that the predisposing cause to the subsequent infection with the bacteria is the existence on the surface of the valve or auricular wall of fresh, unhealed verrucae or plaques. In the preceding chapter I pointed out the primary rôle of the changes in the functional status of the valvular endocardium in the development of endocarditis.

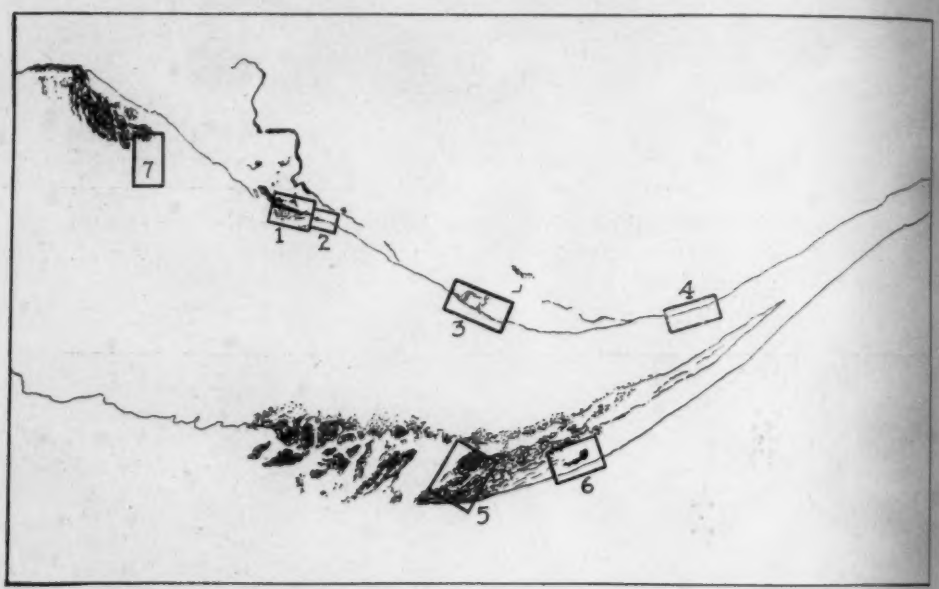

Fig. 29.—A diagrammatic sketch of the valve shown in figure 28 to illustrate the location of the zones shown in figures 30 to 36.

The variability of the bacteriologic findings in cases of endocarditis makes evident the importance of the reaction of the macro-organism to the infection (Falta [29]). It is the greater frequency of the invasion of the blood stream and simultaneous change in the function of tissues receiving the bacteria which is important, and not specific selectivity on the part of the invading organism. The micro-organism should be

27. Swift, H.: Am. Heart J. **3**:12, 1928.

28. Von Glahn, W. D., and Pappenheimer, A. M.: Arch. Int. Med. **55**:173. 1935.

29. Falta, W.: Wien. klin. Wchnschr. **46**:673, 1933

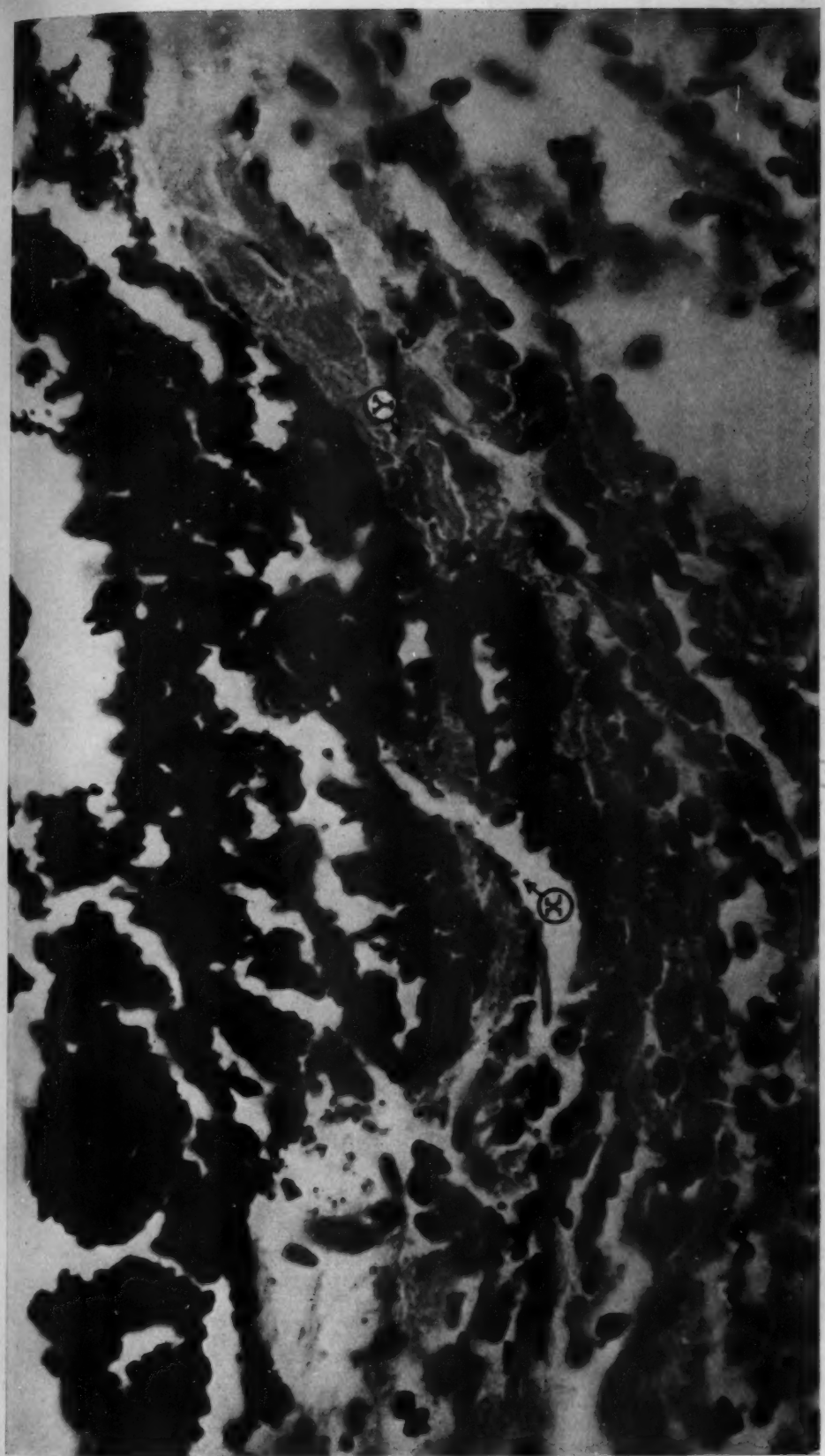

Fig. 30 (zone 1 of fig. 29).—A superficial seminecrotic area of bacterial localization seen as black-stained masses (bacterial clumps) in the endothelial cells of the surface; × 1,200; oil immersion. At *X* and *Y* bacteria have penetrated into the deeper tissues. The cellular infiltration is largely polymorphonuclear leukocytes.

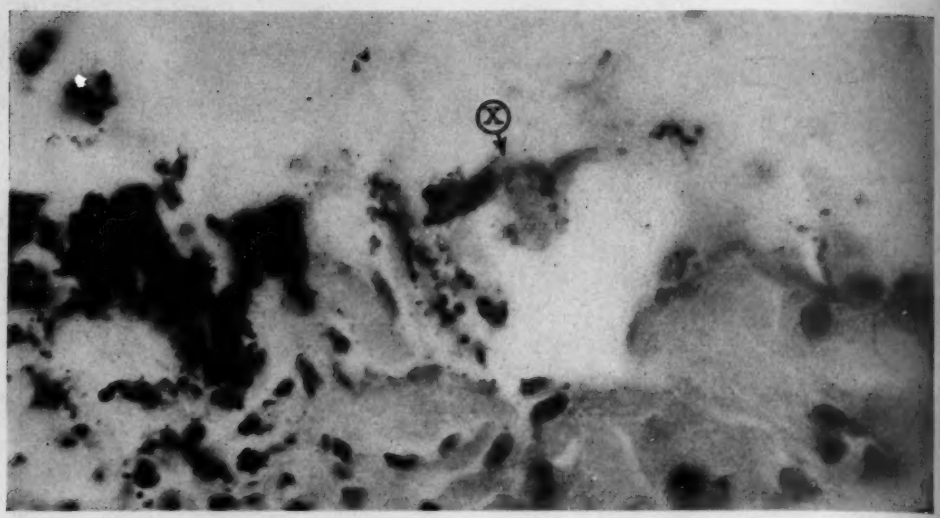

Fig. 31 (zone 2 of fig. 29).—A marginal zone of bacterial infiltration; × 1,200; oil immersion. The necrotic endothelial cells at the left are filled with bacterial masses. There is a partially filled necrotic zone toward the right. At *X* is seen a necrotic endothelial cell partially filled with bacteria.

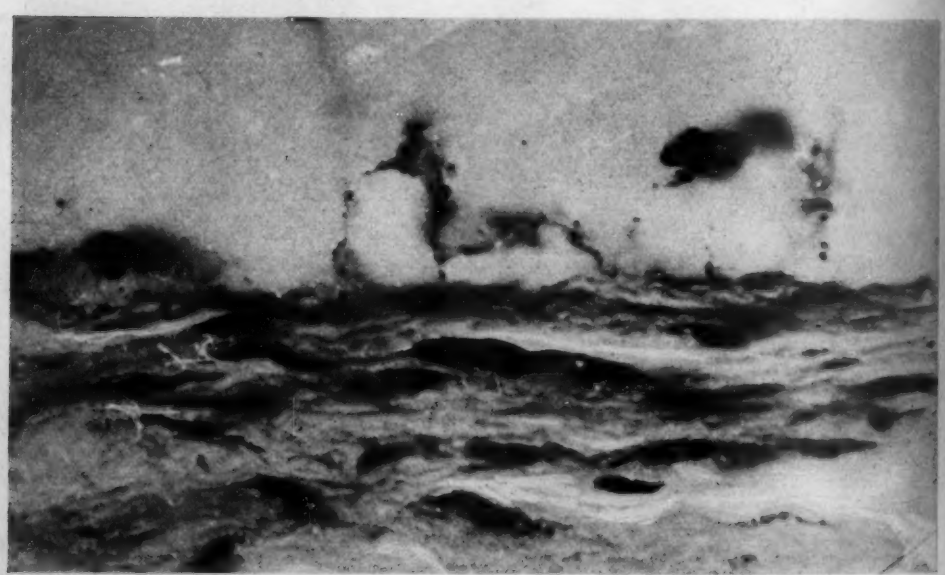

Fig. 32 (zone 3 of fig. 29).—Superficial endothelial cells partially filled with bacteria; × 1,200; oil immersion.

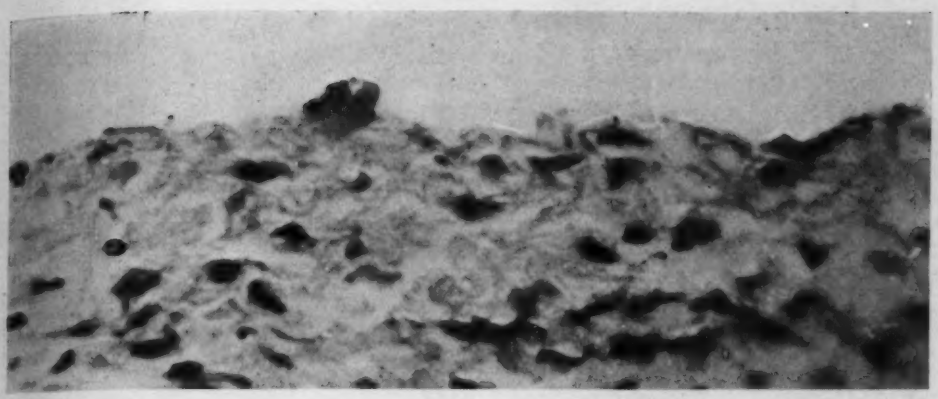

Fig. 33 (zone 4 of fig. 29).—A partially denuded surface with a single endothelial cell partly filled with bacteria; × 1,200; oil immersion.

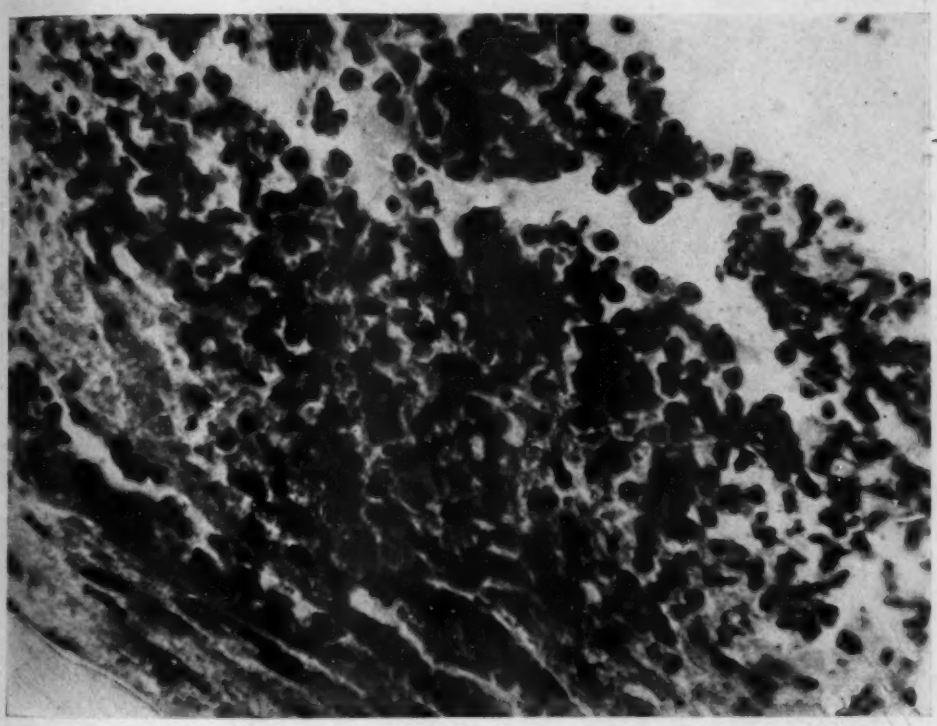

Fig. 34 (zone 5 of fig. 29).—An infiltrated zone from the margin of a necrotic area; × 1,200; oil immersion. An exudate is seen, consisting of polymorphonuclear leukocytes, monocytes and cell débris.

relegated to the background and the conditioning factors that may influence its localization emphasized.

In this connection it is of interest to recall Leschke's [30] statement that septic endocarditis may follow inflammation caused by trauma, freezing or burns of the skin. Such injuries are often associated with marked pressor alterations.

The findings presented here are in agreement with the expressed views and observations. The presented experimental material reveals the early implantation of bacteria on the valvular surface (first a few

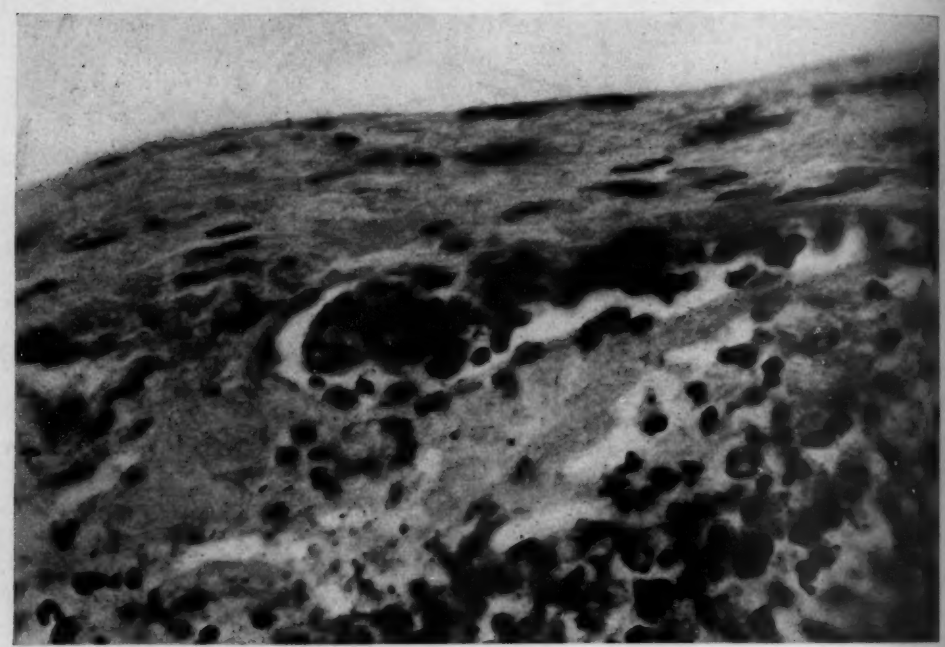

Fig. 35 (zone 6 of fig. 29).—A vascular channel with polymorphonuclear leukocytes and monocytes; × 1,200; oil immersion. The endothelial surface is relatively unchanged.

micro-organisms on the outer layer of the valve, then a mass of bacteria) and their gradual invasion of the stroma of the valve. The change of the endothelial cells due to pressor episodes, presented in its first stages in the first chapter, is to be correlated with the bacterial implantation. The bacteria infect the valves not because of selective properties but, like any other particulate foreign matter, because of the adhesiveness of the damaged valvular endothelium, for particles of india ink are also

30. Leschke, E., in Kraus, F., and Brugsch, T. J.: Spezielle Pathologie und Therapie, Berlin, Urban & Schwarzenberg, 1925, vol. 4, p. 630.

Fig. 36 (zone 7 of fig. 29).—A necrotic portion at the base of the valve showing infiltration of the tissue by bacteria; × 1,200; oil immersion.

picked up by such endothelium. Ultimately ulcerative lesions with the gross and microscopic appearance of the endocarditis seen clinically can be obtained. In some cases repeated injections of pitressin (without injection of bacteria) led ultimately to the development of bacterial endocarditis, the bacteria being of endogenous origin.

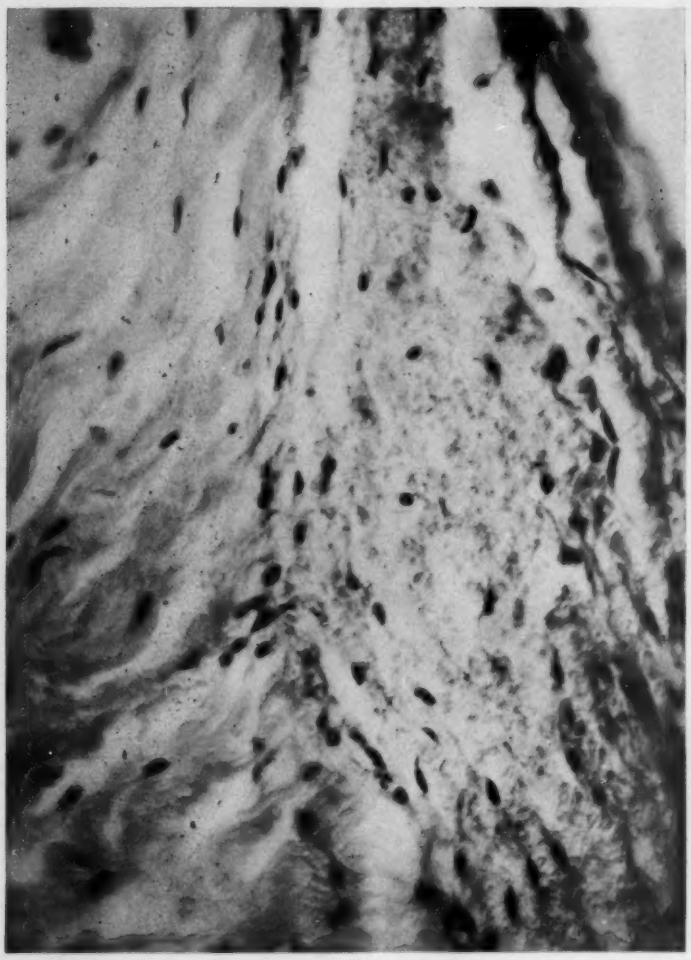

Fig. 37 (dog 3).—A hemorrhagic lesion in a subendothelial area after a pressor episode induced on Dec. 17, 1935; × 520; oil immersion.

The experimental results substantiate my previous assumptions that the pressor episodes cause undue stimulation of the endothelium (in the present case, the valvular, especially the mitral, endothelium). The stimulation is accompanied and followed by changes whereby the endo-

thelium becomes sticky and permeable. This stickiness and permeability permit the bacteria casually present in the blood stream to localize on the valves, with corresponding consequences. But since the pressor episodes include changes in the whole body, the mucous membranes of the respiratory tract are also involved, thus giving an opportunity for bacteria to penetrate the blood stream.

The vast majority of positive results were observed in late winter and spring, although the work was carried on under the same circumstances and with the same technic all the year round.

The animals, which were approximately of the same age and weight, in good health and kept in the same institution, had also the same diet through the year. And yet their reactions to the same amounts of pitressin, given in the same way, showed great variance. In the summer and early fall the animals showed hardly any reactions toward the injections of pitressin. A slight diarrhea of short duration and salivation were the usual symptoms in a vast majority of the cases. Some of the dogs did not react at all.

In the late fall and early winter about 50 per cent of the animals showed the effects of pressor episodes with well pronounced immediate reactions to the injections (severe diarrhea, profuse salivation, vomiting). Some of the animals were prostrated for a long time (up to twenty-four hours).

Late winter and spring were the most favorable times for our experimentations. Then all the animals responded to the injections of pitressin, often violently. Eighty per cent of them showed some pathologic changes, especially on the heart valves (mostly on the mitral valves), the object of these studies. Not uncommonly the injection of a dose of pitressin usually well tolerated in summer caused the death of an animal immediately or in a few hours after the injection. The autopsy revealed great congestion of the heart and pallor and anemia of the visible mucous membranes and gastro-intestinal tract.

From these observations of the reactions of dogs in connection with season, one may also conclude that the change in the endothelium, which is so important for the onset and development of endocarditis, is dual in its origin, being partly the effect of a pressor episode (due to mechanical stimulation) and very likely partly the effect of systemic stimulation associated with season.

The endothelial cells apparently become adhesive. While some of the surface cells to which bacteria adhere may be proliferated mesenchymal elements from subendothelial tissue, it is clear that one can demonstrate histologically changes associated with stimulation (fatigue and necrosis) and adhesiveness as a preliminary stage to bacterial localization.

In conclusion, I may bring out several possibilities in connection with the pressor episodes and localization of bacteria:

1. The bacteria that adhere locally may not grow. If this happens, the whole process subsides, and no bacterial endocarditis can be expected.

2. A pressor episode obviously conditions the valves. Such a pressor episode may be associated with bacterial penetration from the mucous membranes, and thus the same episode may induce both valvular preparation (adhesiveness) and bacteremia (penetration of the mucous membranes and capillary endothelium). Then this pressor episode may lead to development of true bacterial endocarditis.

3. Bacterial penetration from the mucous membranes into the blood stream may have occurred previously, and relatively harmless organisms have been temporarily taken from the circulation and confined to the reticulo-endothelial system. A subsequent pressor episode may, however, result in a flooding of the macro-organism by bacteria which have been previously fixed. If, under such circumstances, the valvular preparation has been adequate and adhesive surfaces are available, these mobilized bacteria then can become localized on the heart valves.

4. The pressor episodes may cause endothelial and subendothelial proliferation and finally scarring, without bacterial adhesion. The healed scar may later easily become stimulated and thus form a nidus for later bacterial localization.

SUMMARY

Bacterial endocarditis may be produced experimentally by inducing pressor episodes in a dog by injections of pitressin followed by an injection of bacteria. This endocarditis closely resembles acute ulcerative endocarditis in man.

The pressor episode simultaneously favors penetration of bacteria into the blood stream and their localization on the heart valve, and thus true bacterial endocarditis due to pressor episodes alone, the bacteria being of endogenous origin, may be observed.

Pressor episodes are more efficacious in producing bacterial endocarditis in late winter and spring.

III. NONBACTERIAL ENDOCARDITIS

From the literature one knows that there is a considerable diversity of opinion concerning the etiology of nonbacterial rheumatic endocarditis and its relationship to bacterial endocarditis.

A large school of thought accepts the thesis that rheumatic endocarditis is caused by some micro-organism. Unable to prove a direct connection between rheumatic endocarditis and any bacterium, the advo-

cates of this theory express the view (MacCallum [31]) that rheumatism is caused by some specific infection not recognizable at present because of lack of technical methods. This school is apparently in agreement that it seems improbable that the numerous bacteria that have been so far isolated in cases of rheumatic lesions have a true etiologic relation to the disease. Fulci [32] found that the action of the bacterial toxins themselves will not lead to the development of endocarditis. The most that can be established is that there is a certain predisposition to the development of the disease.

On the other hand, Siegmund [33] found that the introduction of dyes (as well as colloidal proteins) into rabbits, guinea-pigs and mice leads to proliferation of subendocardial histiocytes to such an extent that a subendothelial layer (*Polster*) is formed. The same may be seen after injections of killed bacteria and after use of vaccines. The histiocytes (Pfuhl [34]), on account of their extremely great affinity to the reticulo-endotheliotropic substances, their superficial (mostly) localization just beneath the thin endothelial layer and their ready destructibility by toxic substances (trypan blue, casein, vaccine), in all probability play an important rôle in the development of endocarditis. De Vecchi,[35] from his experiments, concluded that vital staining (trypan blue) favors the development of changes in inflamed valves, whether of bacterial or of toxic origin. But by no means should one conclude that the dye may exert a true sensitizing (in the general sense) effect. Its action is analogous to that of every other factor contributing to the previous stimulation of the histiocytic system. Eppinger [36] found that treating the rabbits with allylamine, especially in combination with thyroxin, leads to a 100 per cent production of experimental endocarditis resembling human endocarditis lenta.

Eppinger, Kaunitz and Popper [24] in their studies of nonselectivity on the part of bacteria in implantation of these in the tissue found that intoxications from allyl and vinyl compounds, as well as those from acrolein, have a close relationship with serous inflammation. Intoxication from the latter substance is a condition that leads to different pathologic processes (including lesions of the heart valves). Capelli,[37] as a result of his histologic studies of fifty fetuses, concluded that the toxic coefficients play a prominent rôle in the development of such inflammations of valves, which are characterized by proliferation of

31. MacCallum, W. G.: J. A. M. A. **84**:1545, 1925.
32. Fulci, F.: Beitr. z. path. Anat. u. z. allg. Path. **44**:349, 1908.
33. Siegmund, H.: Virchows Arch. f. path. Anat. **290**:3, 1933.
34. Pfuhl: Klin. Wchnschr. **8**:1099, 1929.
35. De Vecchi, B.: Centralbl. f. allg. Path. u. path. Anat. **56**:339, 1933.
36. Eppinger, H.: Klin. Wchnschr. **13**:1630, 1934.
37. Capelli, E.: Pathologica **24**:103, 1932.

histiocytic elements and by degeneration and necrosis of valve tissue. Willer [38] in his studies of toxic endocarditis observed proliferation of histiocytic elements and of endothelium. No bacteria were seen in any of these cases.

Nieberle [39] pointed to the endocarditis developing in horses as a result of artificial immunization and to the endocarditis with erysipelas in swine. This is, in his opinion, a result of an allergic state. Reiter [40] considered rheumatic endocarditis primarily as carditis (originally sub-endocarditis); then, secondly, comes the precipitation from without. The original pathway of the pathologic process is from the inside, and only later does the capillarization of valves take place. He expressed the belief that it is an allergic phenomenon.

Thalhimer [41] found that the similarity of the verrucous lesions in rheumatic and bacterial endocarditides strongly indicates that a simple, fundamental process is the basis of them. Clawson, Bell and Hartzell [42] thought that rheumatic and subacute bacterial endocarditis are caused by the same organism. Bacterial endocarditis, they stated, is a more intensive inflammation than the rheumatic, and the lesions of acute bacterial endocarditis do not differ in any essential respect grossly or microscopically from those of the subacute bacterial type. They also pointed out that it is convenient to distinguish rheumatic and bacterial vegetations, although there are no fundamental differences, bacterial endocarditis being only a more intense inflammation than the rheumatic (Clawson and Bell [43]). Von Albertini [25] found that essential differences between rheumatic endocarditis and endocarditis lenta may reflect manifestations of the defense power of the macro-organism toward the injurious agent (micro-organisms). Of course, the pathogenicity of the micro-organisms must also be taken into consideration. Von Glahn and Pappenheimer [28] thought that active rheumatic vegetations are a necessary and practically constant prerequisite for the implantation of bacteria.

Poynton [44] thought that subacute bacterial endocarditis is not a special disease but a phase of cardiac infection resulting from various causes, one of the most important being the rheumatic. It is accompanied by all the characteristic symptoms of the accidents of the former,

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38. Willer, H.: Centralbl. f. allg. Path. u. path. Anat. **56**:1, 1932.

39. Nieberle, K.: Tierärztl. Rdsch., 1931, pp. 863 and 881; abstr., Centralbl. f. allg. Path. u. path. Anat. **57**:136, 1933.

40. Reiter, C.: Med. Klin. **30**:1719, 1932.

41. Thalhimer, William: Arch. Int. Med. **30**:321, 1922.

42. Clawson, B. J.; Bell, E. T., and Hartzell, T. B.: Am. J. Path. **2**:193, 1926.

43. Clawson, B. J., and Bell, E. T.: Am. J. Path. **2**:469, 1926.

44. Poynton, I. J.: Brit. M. J. **2**:306, 1920.

such as the embolic phenomena, but no suppurative process and no fever. In its outward appearance it passes for rheumatic endocarditis unless the characteristic symptoms are sought for diligently. Murray and Longheed [45] thought that preceding the occurrence of the subacute bacterial endocarditis there is a longer or shorter period of bacteremia during which the complaints are indefinite. The circulating organism gains a foothold on a valve of the heart. This may occur on a valve which was previously thickened as a result of old rheumatic or syphilitic inflammation. Hassencamp [46] brought out that endocarditis lenta is apparently more common since the World War. It is possible, he stated, that articular rheumatism, verrucous endocarditis, ulcerative endocarditis and endocarditis lenta are in close interrelationship, being different manifestations of the same disease. Different persons, having variable properties of defense, show different clinical entities. One is rather inclined to establish different types of diseases, forgetting that there are no diseases; there is merely the expression of a response to environment.

Murray [47] suggested that in the soldier the strain of carrying a heavy pack or the long-continued emotional stimulation, both of which are inseparable from active service, gives rise to excitation of the endocrine system, with lower resistance in the heart valves, making them more susceptible to bacterial invasion. Subacute bacterial endocarditis is a fairly common cause of death in those who have old defects of the cardiac valves. Usually there is an interval of several years between the occurrence of the rheumatic and that of the bacterial infection, but they may follow one another closely.

Coombs [48] thought that the infecting agent in endocarditis is comparatively unimportant. He had seen patients in whom fatigue and privation had seemed to predispose definitely to the development of subacute bacterial endocarditis.

Finally, it is of interest to mention findings of Hertel.[49] She found that owing to long-continued increase in blood pressure and also owing to different injuries of the heart muscle from different causes there appears diffuse thickening of the endocardium with increase of elastic tissue.

It was shown previously that the pressor episodes induced in dogs by intravenous injections of pitressin caused certain changes in the endothelium of the heart valves, especially in the mitral. These changes were of such kind that the endothelium of the valves became sticky

45. Murray, L. W., and Longheed, J. W.: Canad. M. A. J. **2**:666, 1921.
46. Hassencamp, E.: Deutsche med. Wchnschr. **48**:1638, 1922.
47. Murray, L. M.: Ann. Clin. Med. **1**:18, 1922.
48. Coombs, Carey: Brit. M. J. **2**:306, 1920.
49. Hertel, Maria-Pia: Frankfurt. Ztschr. f. Path. **24**:1, 1920.

and permeable, and, if at the time of these changes there was bacteremia, the bacteria, in many cases, localized on the valves, and true bacterial endocarditis developed. The mere initiation of pressor episodes, without the injection of bacteria, in some cases (though rare) led to the development of bacterial endocarditis, the bacteria being of endogenous origin. In the vast majority of cases in which pitressin was given alone, the dogs, after immediate reactions, fully recovered and showed no signs of illness. But in killed animals macroscopic changes were seen, varying from the pinpoint-sized hemorrhage to a thickening of the valve in toto and formation of verrucae, similar to the ones that are characteristic for rheumatic endocarditis.

After pressor episodes the initial changes in the endothelium of the valves and the macroscopic findings were the same in the cases which represented the first stages of bacterial endocarditis and in cases which showed absence of bacteria and signs of healing (with scar formation and rheumatic-like verrucae). The changes observed were not bound to the endothelial layer alone. The subendothelial tissue showed pronounced changes.

Here I present observations of the changes in the subendothelial tissue in cases in which pressor episodes were induced without subsequent injection of bacteria. The changes are shown, step by step, from hours after the single injection of pitressin to periods of months during which the pressor episodes had been repeatedly induced but no endogenous bacterial implantation observed.

EXPERIMENTS

For this group of experiments only young and healthy dogs were used. The pressor episodes were induced by intravenous injections of pitressin. The number of injections and the doses are shown for each of the animals described. After variable lengths of time the dogs were killed by bleeding, the mitral valves were taken for sectioning, and slides were examined.

Some of the findings are presented here. They show the changes in the subendothelial layers, from early manifestations after the pressor episodes to the final development of vegetations and scar tissue. In all experiments described here no bacteria were found, and the whole process proceeded aseptically.

Dog 3.—A healthy young male dog, about 12 pounds (5.4 Kg.) in weight, received a single intravenous injection of pitressin (1 cc.). In seven hours the animal was put to death and the mitral valve examined microscopically.

Figure 37 shows a hemorrhagic lesion in the subendothelial area. Some of the erythrocytes are intact, but most of them are disintegrating. At the margin of the lesion and farther out one observes the appearance of round cells. The surrounding tissue is swollen and edematous. Macroscopically, this lesion appeared as a cherry-red dot the size of a pinpoint.

Figure 38 (same slide) shows a swollen, edematous valve with fibroblastic proliferation in an initial stage. The fibroblasts gradually change their position

from one parallel to the valvular surface to one that is perpendicular to it. They extend to the endothelial surface—in the present case, mostly on the auricular side. Single scattered round cells also can be observed.

Figure 39 (same slide) shows edema and hydration of the tissues, with dilatation of vascular channels, occasional presence of polymorphonuclear leukocytes and ultimate proliferation of connective tissue elements.

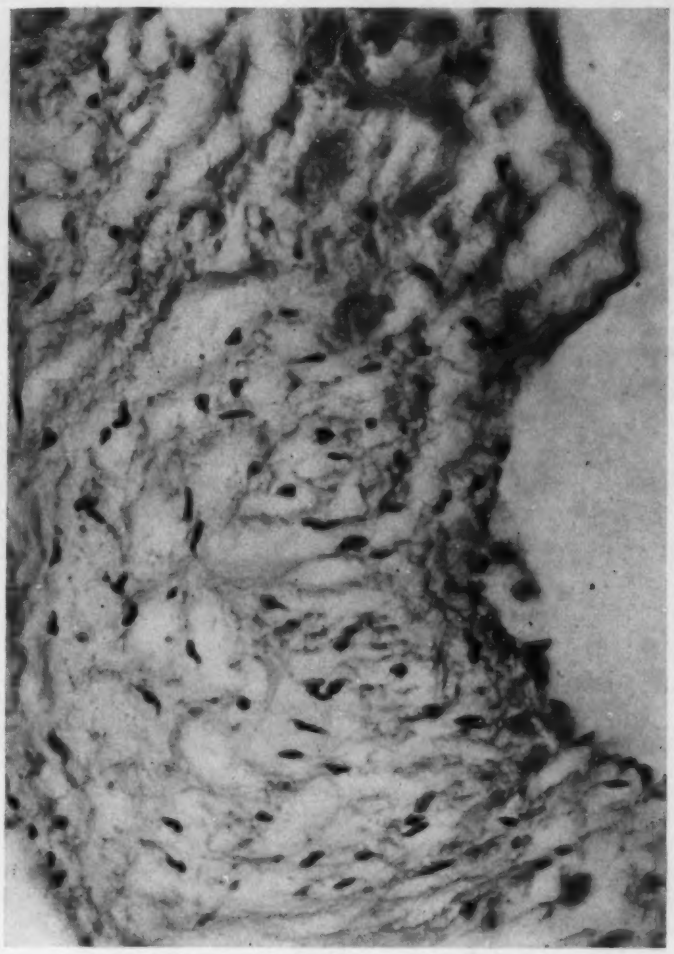

Fig. 38 (dog 3).—A swollen, edematous valve with a fibroblastic proliferation in an initial stage; × 520; oil immersion.

Figure 40 (same slide) reveals proliferating subendothelial cells in the swollen and edematous tissue. The nuclei of some of the fibroblasts have their axes perpendicular to the valvular surface. Single mononuclear cells are scattered all over the field. In the center, around the blood vessel, there appears a nodule

composed of some spindle-shaped cells but mostly of round mononuclear cells. Among them may be seen large cells, each with a large round nucleus, and also cells with vesicular nuclei.

Dog 6.—A young healthy male dog, about 10 pounds (4.5 Kg.) in weight, received injections of pitressin at 9 a. m., 11 a. m., 1 p. m. and 3 p. m. Doses were 0.5, 0.65, 0.75 and 1 cc. The animal was disposed of at 4 p. m. the same day. The endothelial changes in the mitral valve of this animal were described previously.

Figure 41 presents edematous tissue with a large number of scattered round and spindle form cells. Above, beneath the valvular surface, there is a large congregation of cells resembling a nodule. The cells concerned are small and

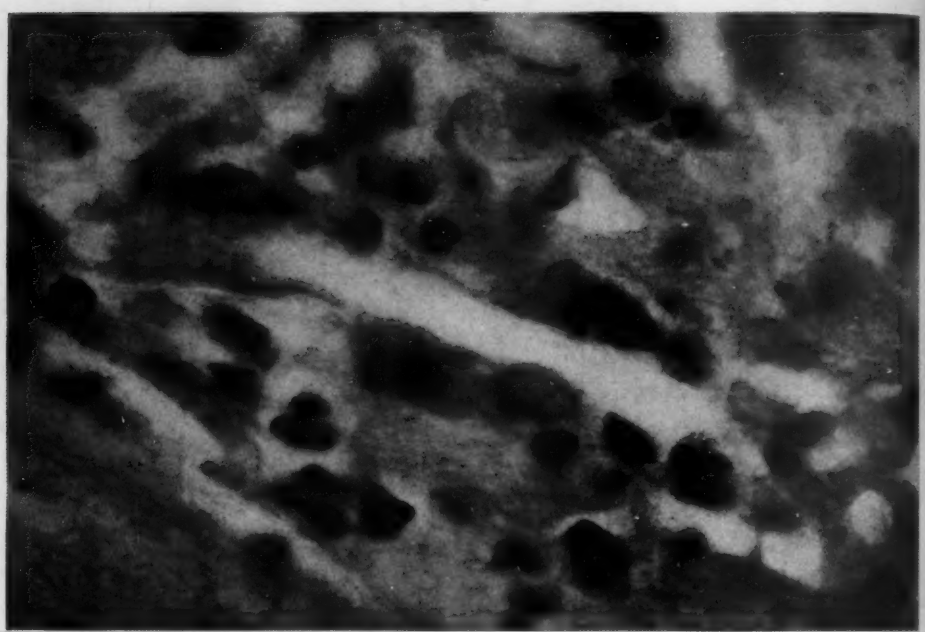

Fig. 39 (dog 3).—Subendothelial tissues showing characteristic hydration and edema after a pressor episode; × 1,500; oil immersion.

large mononuclears and fibroblasts, and some of the cells have vesicular nuclei. The elongated nuclei of a number of the fibroblasts show a tendency to become perpendicular to the surface.

Dog 7.—A young healthy male animal, about 9 pounds (4.1 Kg.) in weight, received four intravenous injections of pitressin at two hour intervals. The doses were 0.5, 0.65, 0.75 and 1 cc. The animal was disposed of in twenty-four hours after the last injection of pitressin. The endothelial changes in the mitral valve of this dog were described previously.

Figure 42 presents the advanced stage of the proliferation of fibroblasts of the valvular tissue. The fibroblasts form a typical palisade perpendicular to the valvular surface. On the left, the fibroblasts have proliferated above the valvular

surface in a manner resembling the initial shape of a verruca. Single round mononuclear cells are seen scattered among the fibroblasts. The valvular tissue beneath the surface appears swollen, edematous.

Dog 8.—A young healthy male animal, about 13 pounds (5.9 Kg.) in weight, received four injections of pitressin intravenously at two hour intervals. The

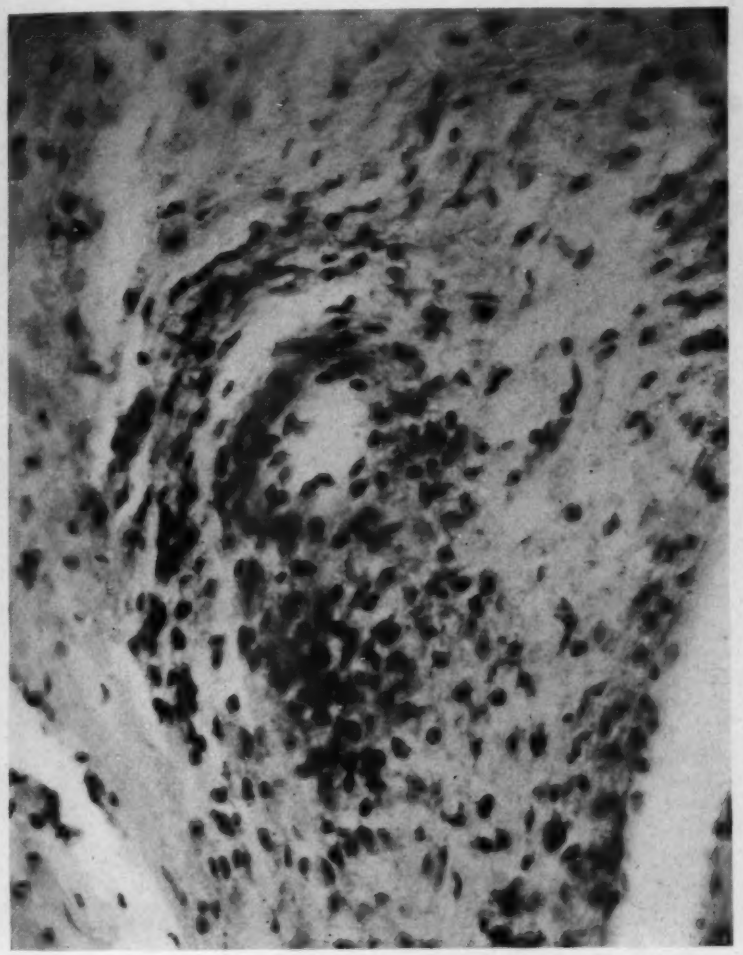

Fig. 40 (dog 3).—A nodule around a blood vessel, composed of some spindle-shaped cells but mostly of round mononuclear cells; × 520; oil immersion.

doses were 0.5, 0.65, 0.75 and 1 cc. The dog was disposed of in forty-eight hours after the last injection of pitressin. The endothelial changes were described previously.

Figure 43 presents a portion of the mitral valve close to the muscle of the base of the valve. The proliferation of interstitial tissue is pronounced, and

there can be observed a large number of round mononuclear cells and fibroblasts. In the center is shown a small nodule composed mostly of mononuclear cells. Some cells are large, with vesicular nuclei. The nodule is located near the capillary blood vessel.

Fig. 41 (dog 6).—Beneath the valvular surface is seen a congregation of cells, resembling a nodule; × 520; oil immersion. The cells are fibroblasts, small and large mononuclears and cells with vesicular nuclei. Pressor episodes were induced on March 2, 1936.

Dog 23.—A young healthy male animal, approximately 11 pounds (5 Kg.) in weight, received on the first day four intravenous injections of pitressin at two hour intervals. On the seventh day, this dog received a second series of injections of pitressin—the same number and at the same intervals of time. The doses

were 1, 1.5, 2 and 2 cc. On the fourteenth day, the animal received a single injection of pitressin, the dose being 2 cc. The dog was disposed of on the twenty-sixth day.

Figure 44 presents the formed scar tissue. Shadows of nuclei of fibroblasts still show their previous palisade formation, with the nuclei directed toward the

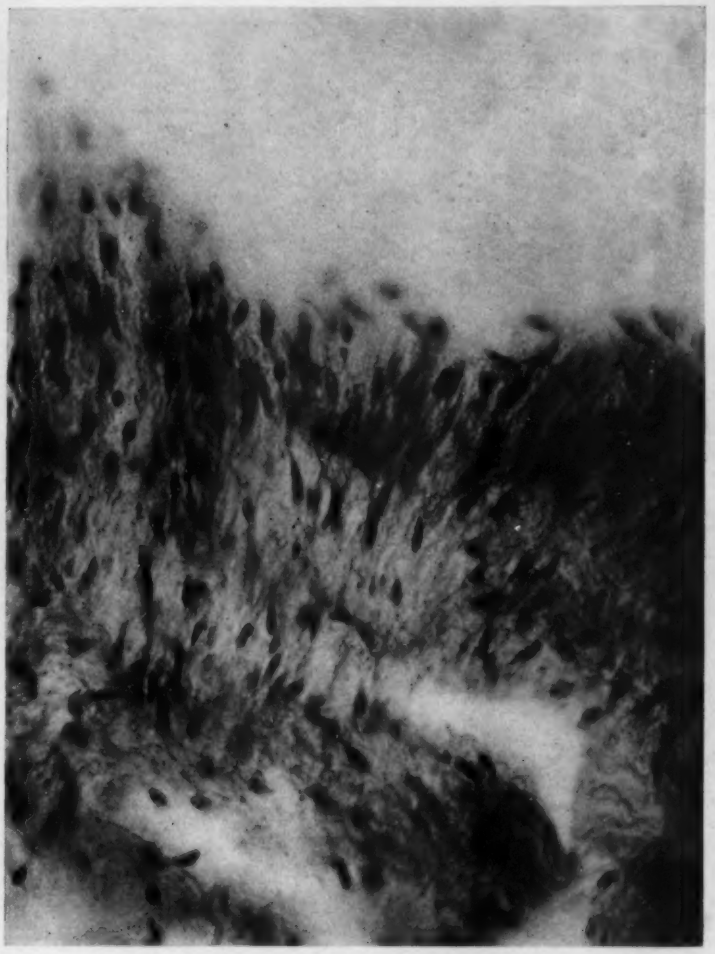

Fig. 42 (dog 7).—Advanced proliferation of fibroblasts, forming a typical palisade, perpendicular to the valvular surface; × 520; oil immersion. On the left is seen a proliferation of fibroblasts above the surface, resembling the initial shape of a verruca. Pressor episodes were induced on March 2, 1936.

valvular surface. The congregation of scar tissue on the surface in the form of a small verruca-like formation shows its development directly from fibroblasts from within the valve.

Figure 45 (the same specimen, another slide) reveals one of the initial stages of the formation of a verruca (compare fig. 42). The fibroblasts in palisade formation and perpendicular to the valvular surface have bulged out of the valvular stroma and formed a leaflike body on the surface. The nuclei of the fibroblasts are well seen. On the right side of the leaflet the endothelium, with nuclei of a cubic form, can be observed, while on top and on the left it is entirely absent.

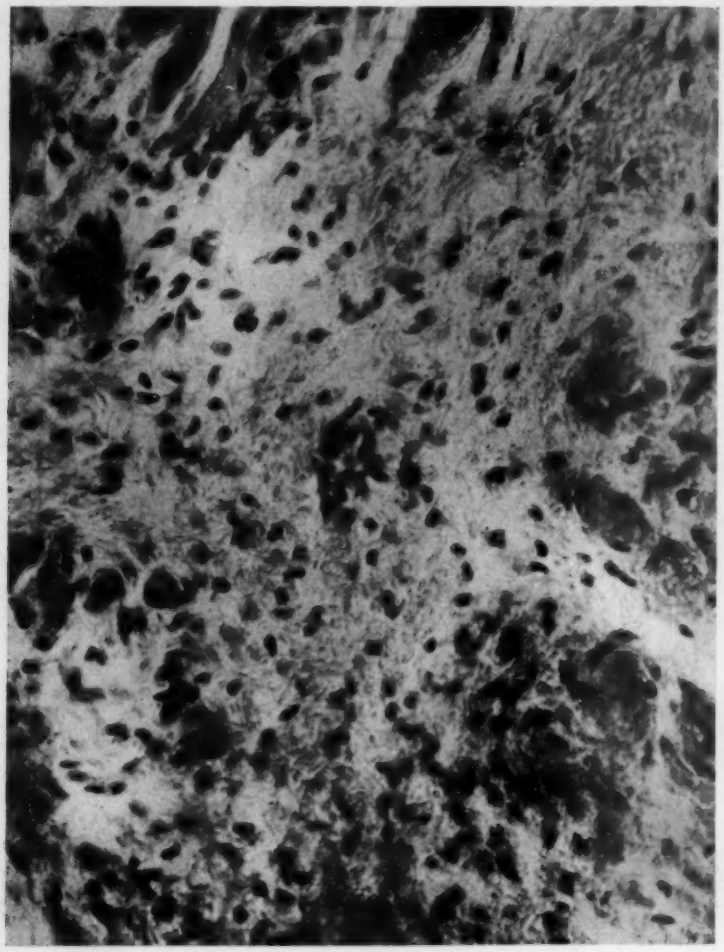

Fig. 43 (dog 8).—In the center, near the blood vessel, is a small nodule composed mostly of mononuclear cells; some of the cells are large, with vesicular nuclei; × 520; oil immersion. Pressor episodes were induced on March 2, 1936.

On the top the cell proliferation is still in an active stage, while below it is subdued. At the base there are unchanged fibroblasts. Single scattered round cells are observed.

Dog 24.—A young healthy male animal, about 10 pounds (4.5 Kg.) in weight, received on the first and fifteenth days three injections of pitressin of 0.5 cc. each.

three hours apart. On the forty-third day, the dog received another series of three injections of pitressin, the dose being 0.75 cc. and the interval between injections three hours. On the fifty-seventh day, this animal received an additional three injections of pitressin at three hour intervals. The dose was 1 cc. The dog was disposed of on the seventy-fifth day.

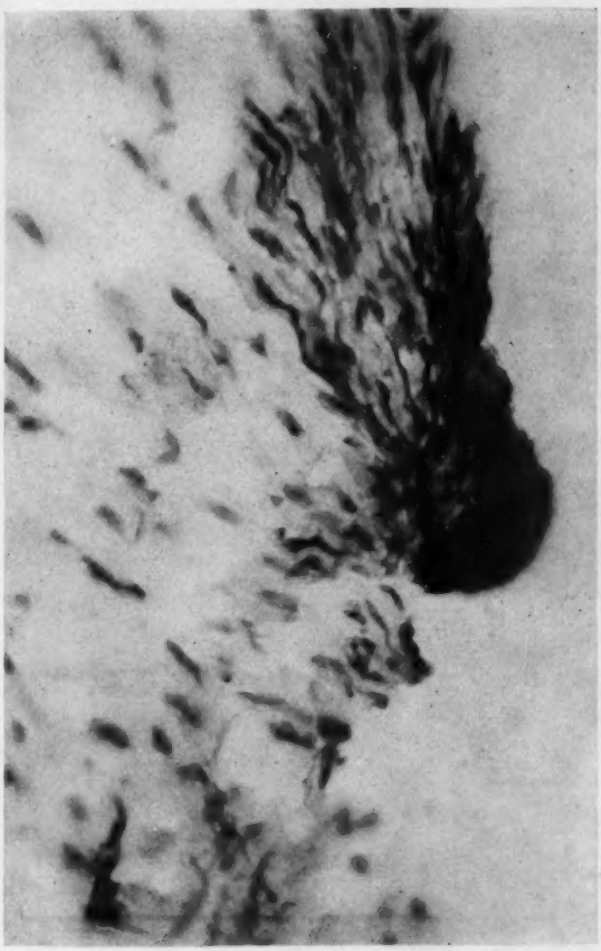

Fig. 44 (dog 23).—Scar tissue formed from fibroblasts, which still show their previous palisade formation and are congregated on the surface in the form of a small verruca-like formation; × 520; oil immersion. Pressor episodes were induced repeatedly on March 19 and 26 and April 3, 1936.

Figure 46 presents an area of scar tissue in the mitral valve. Bundles of this tissue are seen well, extending from the deeper tissue of the valve to the surface. Some of them are parallel to the valvular surface. There are areas with no formed elements, and occasional round cells are observed. The surface is covered with endothelial cells of more or less normal appearance.

Figure 47 (the same slide) shows the formation of a small verruca. The lower center portion is composed of fibrous tissue with few, if any, formed elements. The upper surface of the formation is denuded of the endothelial cover, and on the right side there is a conglomeration of cells with round and oval nuclei, poorly stained. At the base are bundles of scar tissue.

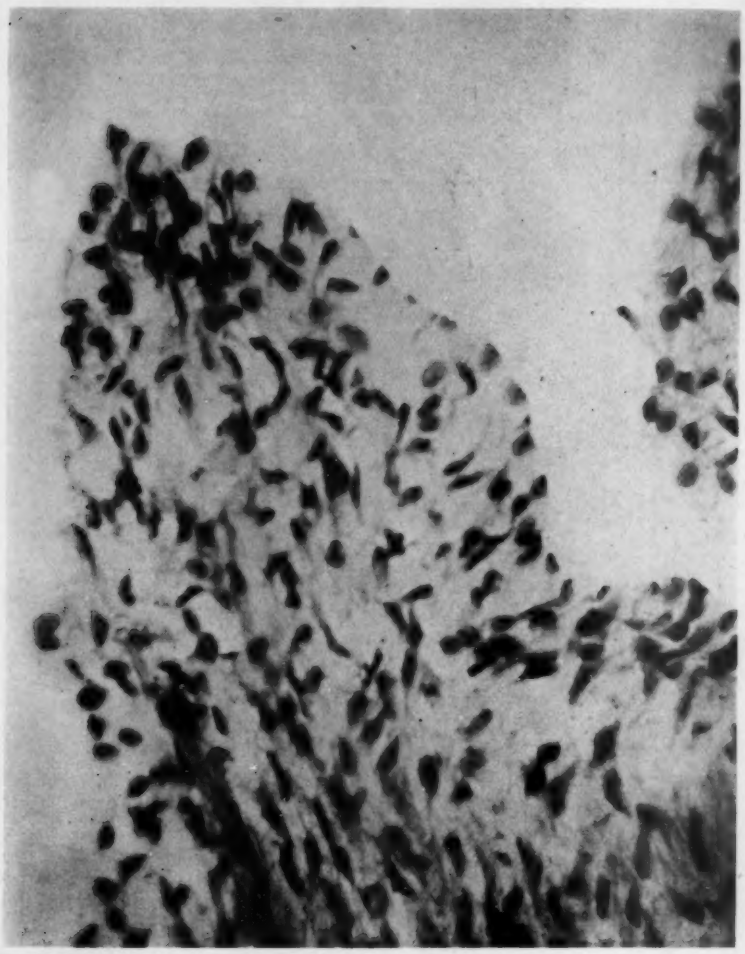

Fig. 45 (dog 23).—A leaflike body on the surface, formed by bulging out of the fibroblasts of the valvular stroma; × 520; oil immersion. On the top the cell proliferation is still in an active stage. On the right side of the leaflike body the endothelial cells, with nuclei of cuboid form, are preserved.

Figure 48 (the same slide) presents a small nodule in the deep tissue of the valve, not far from the distal end of the latter. The nodule is composed mostly of mononuclears. Some of these are large, with poorly stained large vesicular nuclei, round and irregularly shaped. Occasionally one observes here a large cell

with two nuclei, poorly stained. At the upper edge of the nodule and on the right side of it are capillaries. A dividing cell is to be observed below.

Figure 49 (same specimen, but different slide) presents a small verruca, composed of fibrous tissue. In the lower portions of the photomicrograph scar tissue is well seen, especially on the left. Just above the latter a small blood vessel is

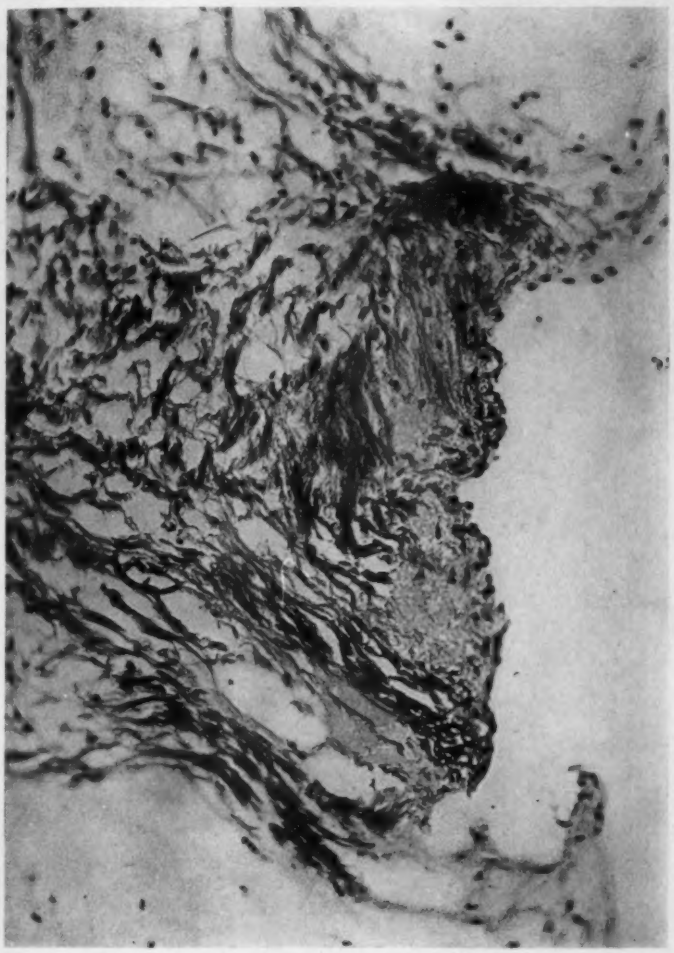

Fig. 46 (dog 24).—An area of scar tissue in the mitral valve, with bundles of scar tissue extending from the deeper tissue of the valve to the surface and some parallel to the valvular surface; × 520; oil immersion. The valvular surface is covered with endothelial cells. Pressor episodes were induced on May 1 and 15 and June 12 and 26, 1936.

shown. The surface of the formation is covered with endothelial cells. A few scattered round cells are seen.

Dog 25.—A young healthy male animal, about 12 pounds (5.4 Kg.) in weight, received on the first and on the fifteenth day three intravenous injections of

pitressin at three hour intervals, each dose being 0.5 cc. On the forty-third day, three injections of pitressin were given at three hour intervals, the doses being 0.75 cc. each. On the fifty-seventh day, another three injections of pitressin were given at three hour intervals, each dose being 1 cc. On the seventy-eighth day, the animal received an additional three injections of pitressin. The doses were 1, 1 and 2 cc., with a three hour interval between injections. On the eighty-third day, the animal was disposed of.

Fig. 47 (dog 24). A small verruca with bundles of scar tissue at the base; × 520; oil immersion.

Figure 50 presents a well developed papilla-like verruca. The mass of it consists of fibrous connective tissue with scarce nuclei. The surface is covered with an endothelial layer, mostly of normal appearance, though some of the nuclei appear swollen and are poorly stained. At the surface more cell elements and

some polymorphonuclear leukocytes are seen. There are a few newly formed capillary blood vessels, and beneath the valvular endothelium hyaline masses are observed.

Pressor episodes artificially induced by injections of pitressin cause certain changes in the heart valves. These changes involve the subendothelial structure as well as the valvular.

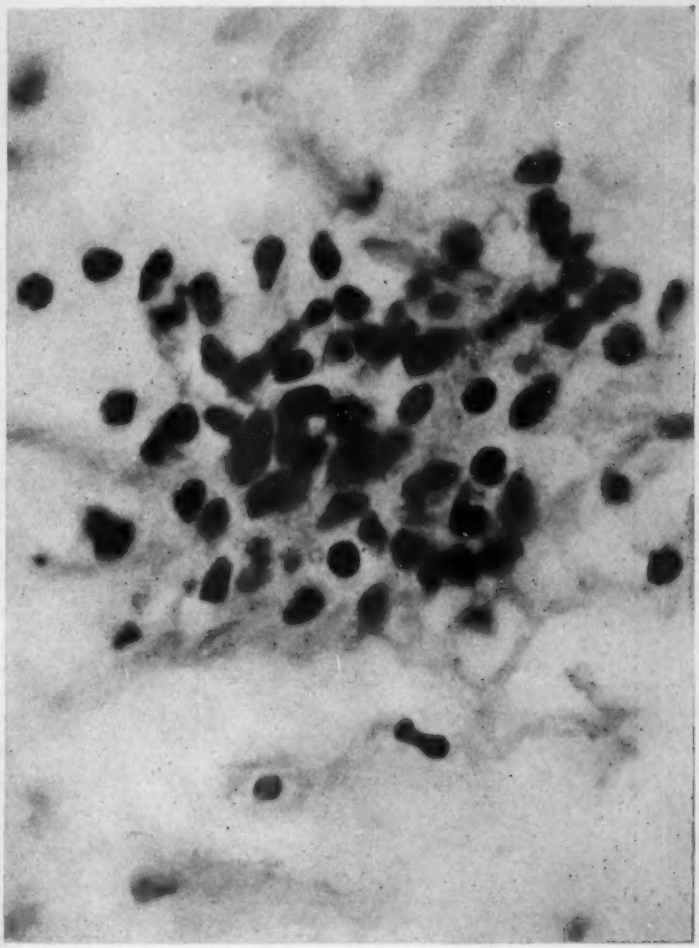

Fig. 48 (dog 24).—A small nodule in the deep tissue of the valve, near its distal end, composed mostly of mononuclears, some of which are of large size, with poorly stained large vesicular nuclei of round and irregular shape; × 520; oil immersion.

As early as a few hours after the induction of pressor episodes, the valves appear macroscopically swollen, edematous. On the surface

one may find small lesions (as red spots), the remains of minute subendothelial hemorrhages.

In the microscopic picture, the fibroblasts begin to proliferate very actively and gradually assume a position perpendicular to the valvular

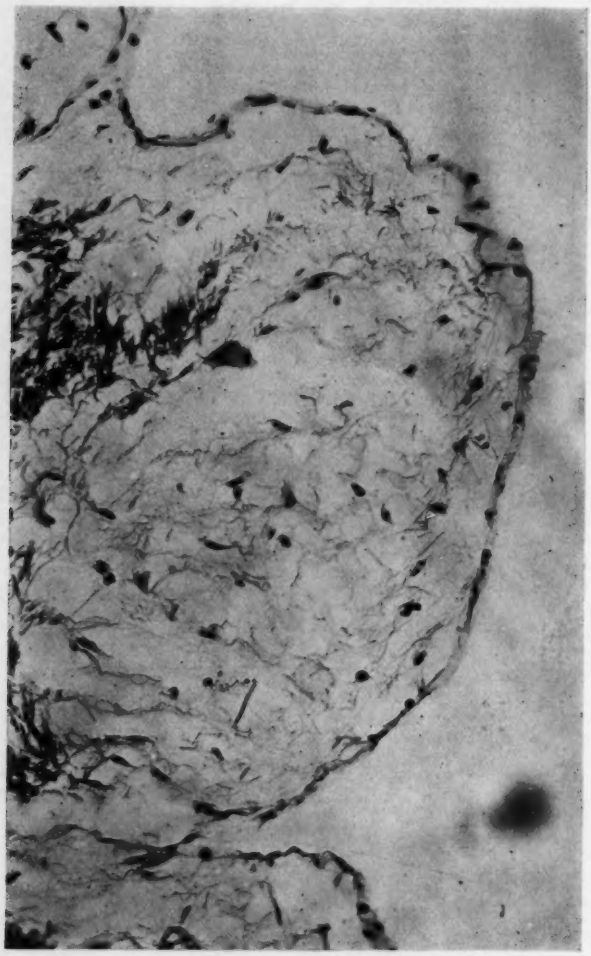

Fig. 49 (dog 24).—A small verruca composed of fibrous tissue, with a well marked area of scar tissue in the lower portion; × 520; oil immersion. The surface of the formation is covered with endothelial cells.

surface; i.e., they form palisades. In some places these fibroblasts proliferate beyond the valvular surface, when they form a base for verrucae. Vascularization begins, and one sees a true verruca, mostly covered by endothelium.

The fibroblasts forming the palisades, with time, also form scars. These scars may be of different size and location. They may come close to the surface, entirely changing the normal appearance of this affected part of the valve. The scar tissue may also extend above the valvular surface in such a manner as to resemble a small verruca. It

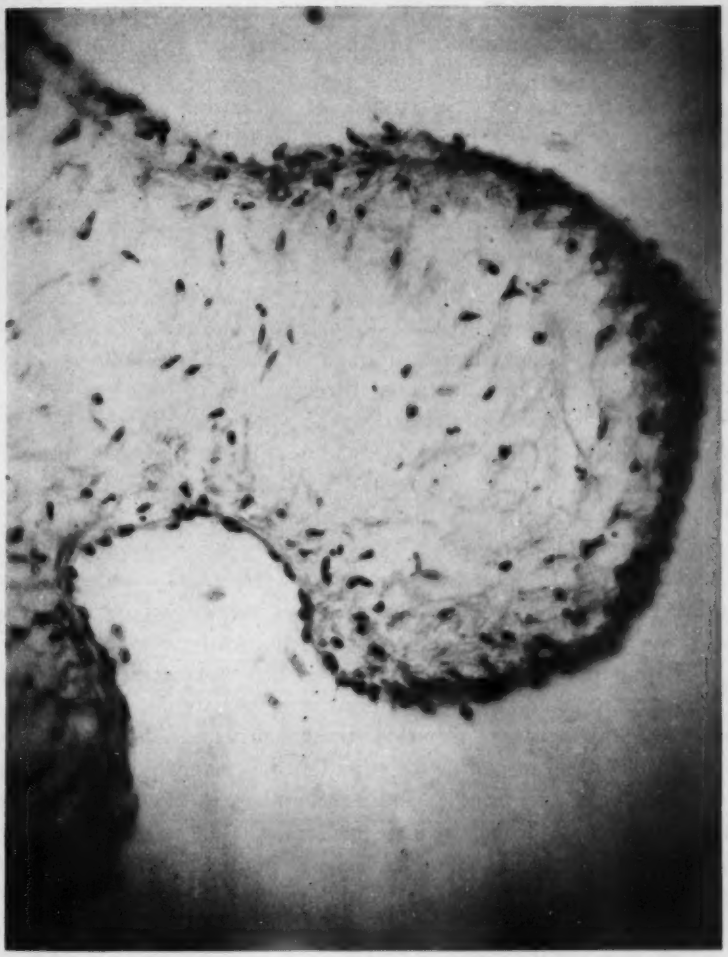

Fig. 50 (dog 25).—A well developed papilla-like verruca, consisting of fibrous connective tissue with few nuclei; × 520; oil immersion. Newly formed capillary blood vessels are noted, as well as some polymorphonuclears at the surface. The endothelial layer covers the whole surface, with hyaline masses beneath it. Pressor episodes were induced on May 1 and 15, June 12 and 26 and July 17, 1936.

also may be seen at the base of the formed verrucae and also scattered all over in the deeper tissue of the valve. In other words, the fibroblasts,

wherever they appear (as a result of induced pressor episodes), in due time form dense fibrous tissue, which characterizes the stage of repair from the injury that has been inflicted on the valve.

One also observes the appearance of round cell infiltration. The single mononuclears are seen scattered over the injured tissue as well as gathered in small nodules. These do not exactly resemble the Aschoff bodies of the heart muscle, but, if due allowance is made for the differences in histologic structure of the valve, one may consider them analogous.

Jaffé,[20] from comparative studies of numerous cases of malignant endocarditis and of the heart valves of patients who had septicemia without grossly visible endocardial changes, observed rapid proliferation of the subendothelial fibrocytes, which, near the surface, arranged themselves in palisades. Leary [50] studied the early phases of rheumatic endocarditis in persons who came to violent deaths while in apparent health or who died suddenly without hospitalization. He found that the characteristic lesions in valves of persons suffering from rheumatic endocarditis show the formation of a defensive palisade of cells on their contact edges. He illustrated three phases of this reaction: first, one before the formation of a verruca, with bacteria on the surface of the palisade; second, formed verruca without demonstrable micro-organisms; third, mucoid (Leary) degeneration of the palisade in an old scarred valve, without micro-organisms. Eppinger, Kaunitz and Popper [51] reproduced in dogs experimentally a picture of rheumatic endocarditis. They obtained it by inducing serous inflammation (entirely excluding the allergic state) by means of poisonous chemical dyes. The changes, as in the present case, were seen mostly in the mitral valve and consisted of edematous thickening of the valve with visible "blood dots." Microscopically, single scattered or aggregated erythrocytes could be observed not far away from the valvular surface. They also observed multiplication of large cells with round nuclei, corresponding to histiocytes and fibrocytes with highly extended and small nuclei, which formed bundles or buttons projecting into the lumen. They were covered with endothelium. In other portions of the subendothelial layers these fibrocytes multiplied and appeared in palisade form. The most essential fact is that they finally formed fine papillar elevations on the valvular surface, which in general resembled small warts. In the valve small nodules resembling the Aschoff bodies of myocardial rheumatism were observed. These findings led the authors to the conclusion that all the described changes were due to poisons that acted on the permeability of the capillary membrane.

50. Leary, T.: Arch. Path. **13**:1, 1932.

51. Eppinger, H.; Kaunitz, H., and Popper, H.: Die Seröse Entzündung, Vienna, Julius Springer, 1936.

Induced pressor episodes such as I have described evoke in a valve a pathologic picture the same as that just described. The changes in pressor levels of the blood circulation lead to anoxemic states with considerable nutritive change in certain tissues (Petersen [1]). The mechanical stimulation of the heart valves, especially of the mitral valve, owing to its anatomic position and physiologic rôle, is a base for reflection of vasomotor changes due to changes in pressor levels in the circulation. This is proved by the action of pitressin in these experiments.

The fundamental and initial changes in the heart valves of animals with nonbacterial endocarditis are the same as those in animals with bacterial endocarditis. After mere pressor episodes, not accompanied by simultaneous bacteremia, the heart valves show all stages of pathologic changes resembling those seen in rheumatic endocarditis. The appearance of micro-organisms in the blood with their subsequent localization on the prepared surface of the valve changes nonbacterial (rheumatic) into bacterial endocarditis.

COMMENT

It is generally agreed that a damaged valve will be more susceptible to later afflictions. Clinicians lately detect more damaged heart valves than previously. They also agree that many cases of endocarditis escape detection, and observations such as those of Leary [50] substantiate these statements. Rothschild, Kugel and Gross [52] in a study of autopsy material from 161 cases of rheumatic cardiovalvular disease stated that it is striking to note the high grade mechanical defect existing in persons living even to the fifth and sixth decade with little or no evidence of congestive heart failure.

It seems reasonable on account of clinical and experimental evidence to conclude that all types of endocarditis have the same origin and that this lies in the human body itself. Any infectious agent, before it localizes on the valve, must find a foothold, which is presented either in congenital pathologic change of the valve through previous valvular disease or in injury to the endothelium. The injury to the latter is accompanied simultaneously with changes in the subendothelial layers of the valves. It may be induced (as mentioned previously in the review of the literature) by different chemical substances, vaccine and other agents, and, as in the cases described here, by pressor episodes. But if no infection is present (which is most frequently the case) the valve undergoes regenerative changes, leaving quite often no detectable clinical

52. Rothschild, M. A.; Kugel, M. A., and Gross, L.: Am. Heart J. **9**:586, 1934.

signs. This accounts for overlooked cases of rheumatic injury to the heart valves and findings of old lesions in cases of acute endocarditis post mortem.

The elevations of blood pressure levels, i. e., the manifestation of the pressor episodes, should be distinguished in their origin. Those of which I speak here are reflections not of a mere increase in the output of the heart, increase in blood volume, increase in the viscosity of the blood or decrease in the elasticity of the arteries but of a characteristic status in the vascular bed, particularly in the small arterioles and capillaries. The constrictions of the latter cause the anoxemic stimulation in corresponding tissues, and the endothelium of the small arterioles and capillaries becomes permeable and sticky. It is not the elevation of the blood pressure but the cause of it that is important in the development of a pathologic status in a certain area.

The contractions (with following dilatations) of the vascular bed may be initiated by many general factors (endocrine, nutritive, toxic) but are most commonly associated with swings in biologic rhythms that result from the efforts of the body to adapt itself to the shifting cyclonic circulation of the atmosphere (Petersen [1]). Injections of pitressin (Kamm and collaborators [53]) cause a rise in blood pressure through stimulating the blood vessels by peripheral action (Geiling [54]), evoking constriction of the finer arterioles and capillaries. This action of pitressin is analogous to the action of meteorological factors (pressor episodes) which one meets daily.

The pressor episodes, which so readily cause injuries to the heart valves (particularly the mitral valve), and the connection of clinical manifestations of endocarditis with age, season and geographic distribution definitely point to the rôle of vascular instability as a fundamental factor in the etiology of endocarditis, bacterial or nonbacterial. It is the vascular net, the nourisher and regulator of the well being of a tissue, that keeps the latter in a normal state. The more unstable this factor is and the more it is influenced by environment, the more the corresponding tissue is liable to be damaged.

Endocarditis is a disease confined largely to the northern latitudes, where meteorological changes (pressor episodes) are so pronounced. It is also a seasonal disease, the late winter and spring being its harvest time, when the macro-organism is fatigued (endothelium is permeable and sticky) and its recovery delayed (Petersen [1]). Young persons more readily succumb to it, and the vascular system of the young person is more responsive to external influences.

53. Kamm, O.; Aldrich, T. B.; Grote, I. W.; Rowe, L. W., and Bugbee, E. F.: J. Am. Chem. Soc. **50**:573, 1928.

54. Geiling, E. M. K.: J. A. M. A. **104**:738, 1936.

CONCLUSIONS

Pressor episodes, in the absence of bacteria, can produce lesions (especially in the mitral valve) which are characteristic of those found in the human heart in rheumatic endocarditis.

The underlying changes in the valve are seen in bacterial endocarditis (until localization and multiplication of bacteria) as well as in the nonbacterial form.

Bland endocarditis is a local reaction of a macro-organism to changes in its vascular status. Bacterial endocarditis may represent a functional stage in which bacterial adhesion and proliferation have been added to the primary change in the endothelial status.

The pressor episodes, which play such a rôle in the etiology of endocarditis, may be conditioned by many factors, such as constitutional vasomotor lability, emotional excitement and meteorological environment.

GENERAL SUMMARY

Pressor episodes artificially induced in dogs with pitressin cause, in many cases, pathologic changes in the heart valves, especially the mitral valve, which closely resemble changes in human cardiac valves in rheumatic and ulcerative endocarditis.

Macroscopically, one may see characteristic valvular changes, varying from swelling and edema of the valve (usually the mitral valve), with cherry-red flat dotlike lesions, to formed scars and verrucae or vegetations and ulcers.

Microscopically, the red dots prove to be hemorrhagic lesions in the subendothelial areas. Around the margins of these lesions round cells are observed. The surrounding tissue swells and becomes edematous; the fibroblasts begin to proliferate. The blood vessels are distended, around some of them the formation of nodules, composed mostly of round mononuclear cells with some spindle-shaped cells, can be observed. In the deeper tissues of the valve are small nodules, composed mostly of mononuclear cells, some of which are of large size, with large poorly stained vesicular nuclei, round and irregular in shape. Occasionally large cells with two nuclei, poorly stained, can be observed. These nodules are always close to capillaries.

The proliferating fibroblasts build a defensive palisade of cells on the edges of valves. In some places these fibroblasts proliferate beyond the valvular surface, forming a base for verrucae. Vascularization begins, and a true verruca is seen, mostly covered by endothelium.

The fibroblasts that appear in response to induced pressor episodes form dense fibrous tissue, characteristic of a stage of repair of the injury inflicted on the valve. This dense fibrous tissue may be found

scattered throughout the deeper tissue of the valve or close to the surface, entirely changing the normal appearance of the affected part of the valve.

The most striking changes seen microscopically concern the valvular endothelial surface. The endothelial cells swell; their nuclei change their form and position. The swelling may continue to such extent that in some places the cells protrude from the surface of the valve into the lumen of the heart. The cytoplasm appears gelatinous and stains poorly; soft protoplasmic masses of the endothelial cytoplasm are often seen, extending far above the vascular surface. With these degenerative changes of the endothelial cells, the valvular surface becomes accordingly roughened and adhesive, so that the bacteria (of exogenous or endogenous origin) in the blood stream may adhere. If the blood is free from bacteria, or if those that are present do not adhere, or if they adhere but do not multiply, healing begins and the process subsides, ultimately leaving verrucae, scars of fibrous tissue in the stroma of the valve, as mentioned.

If the bacteria (from within or from outside the body) adhere and multiply, the development of true bacterial endocarditis begins, which results in ulceration of the valve.

These observations emphasize the status of the vascular bed in the development of endocarditis, no matter of what type this endocarditis may be. Any factor (constitutional vasomotor lability, emotional excitement, meteorological environment and others) which may condition the pressor episodes may lead to the development of bland endocarditis; bacterial endocarditis presents the end-result when bacterial adhesion and proliferation have been added to the primary change in the endothelial status.

Prof. W. F. Petersen gave advice, assistance and encouragement, which made possible the accomplishment of this work.

REACTIVATION OF A PRIMARY TUBERCULOUS COMPLEX AS A SOURCE OF TUBERCULOUS REINFECTION

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The so-called adult type of tuberculous disease is now thought of as the result of an interaction between the tubercle bacillus and the tissues of one who at some time in the past has sustained a primary tuberculous infection with consequent altered reaction of the tissues. The bacilli of reinfection may be exogenous, or they may come from foci of quiescent tuberculosis which are exacerbated by certain deleterious influences, such as intercurrent disease.

In order to demonstrate reactivation, fresh lesions must be found in the neighborhood of foci which give evidence of having been previously quiescent, and other endogenous sources must be excluded. The original focus must show fibrosis, hyalinization or calcification, as indicative of certain, though perhaps temporary, arrest of the disease. It is obviously impossible to state that the old focus may not have continued to be active even with the presence of some degree of cicatrization. The present study is concerned solely with the reactivation of a primary complex or of its subsidiaries and not with the more frequent phenomenon, reactivation of reinfection scars.

A model case for this study, then, would show reactivation of a component or components of an old primary complex without other foci from which this fresh infection could have drained and with demonstrable dissemination from this point of reactivation to produce clinically manifest disease. This dissemination might occur by progressive invasion of the mediastinal nodes until the lymphatic venous angle of the subclavian vein is reached (Ghon and Kudlich [1]). The result would be hematogenous spread with the development either of an isolated organ disease of the lung or true miliary tuberculosis. A parenchymal focus or a diseased lymph node may rupture into the bronchial lumen and so produce a bronchiogenic spread. Pericardial and pleural cavities may be invaded in the same fashion.

From the Department of Pathology, Cleveland City Hospital, and the Institute of Pathology, Western Reserve University.

1. Ghon, A., and Kudlich, H.: Ztschr. f. Tuberk. **41**:1, 1924.

In a previous study[2] several cases were found which could be regarded tentatively as examples of reactivation. Into most of the lungs solution of formaldehyde U. S. P. was injected by way of the pulmonary artery or trachea, and after fixation the lungs were sectioned in the coronal plane. All the nodes which drain the lungs were carefully sectioned in the gross, and several were studied microscopically. Not only the mediastinal but also the pancreaticolienal nodes were examined in this fashion, since the lymph nodes at the head of the pancreas and those extending along the cephalad surface of the pancreas to the hilus of the spleen receive drainage from the lungs. Only complete autopsies were given consideration. The organs were seen by one or both of us personally.

Not all examples of reactivation discovered were model cases. This, however, is not remarkable since the ideal case presents a passing stage of the process of reinfection and can be demonstrated only by a stroke of good fortune. The cases, therefore, represent phases which must be studied sequentially. Such a study cannot offer absolute proof of the existence of reactivation; that the phenomenon occurs can be regarded only as a strongly probable hypothesis.

CASE 1.—A 59 year old Negro presented: myocardial failure; generalized cardiac hypertrophy and dilatation; coronary sclerosis; myocardial fibrosis; thrombosis of the internal iliac arteries; pulmonary embolism; a primary tuberculous complex of the lingula pulmonis and of the left bifurcation and right paratracheal nodes, and chronic active conglomerate tuberculosis of the left upper lobe, in the lingula pulmonis, in the vicinity of the primary focus.

The primary focus was a calcified nodule measuring from 5 to 6 mm. in diameter and situated beneath a subpleural bulla (fig. 1*1*). Immediately contiguous to the primary focus there was no fresh tuberculous process, but 5 mm. distant from it and extending for 6 cm. were nodular caseous masses (fig. 1*2*). These masses were well encapsulated, but there was active progessive disease at many points. The lymph nodes showed calcification and ossification, but no active disease was found in the gross examination.

This case showed a remote primary complex with fresh progressive conglomerate tuberculosis in the vicinity of the primary focus. No other lesions were found. Since the lesions were sharply restricted to exactly the lobules about and in the area of drainage of the primary focus, it is more probable that the active disease was due to reactivation rather than to exogenous reinfection. Tubercles are commonly found along perivascular and peribronchial lymphatics which drain a primary focus and when the primary complex is obsolete are demonstrable as calcified foci. This case can therefore be most reasonably interpreted as one of reactivation of such subsidiary or drainage tubercles. Focal

2. Reichle, H. S., and Gallavan, M.: Arch. Path. **21**:797, 1936.

extension by contiguous growth has occurred, but there is no progressive disease in the lymph nodes or elsewhere in the body.

CASE 2.—A 54 year old white man presented: acute ascending pyelonephritis following suprapubic cystotomy and fulguration of papilloma of the bladder; bronchopneumonia; an obsolete primary tuberculous complex of the upper lobe of the left lung and of the superior bifurcation lymph node; a caseous conglomerate tubercle of the upper lobe of the left lung, adjoining a bronchus with partial obstruction and bronchiectasis.

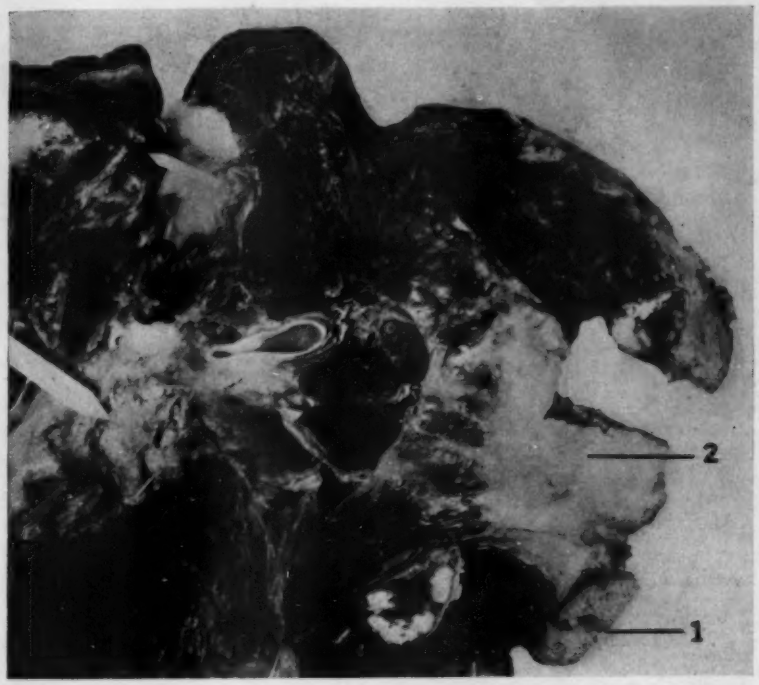

Fig. 1 (case 1).—*1*, subpleural bulla, beneath which is a primary focus. *2*, area of reactivation, a cross-section of which is lying on the upper lobe of the left lung. Arrows point to calcified lymph nodes.

A partially calcified primary focus (figs. 2*1* and 3*1*) 0.5 cm. in diameter was found beneath the pleura in the anterior subapical region of the upper lobe of the left lung. In the line of drainage from the focus to the hilus was an encapsulated caseous mass, which on one side (fig. 2*2*) was anthracotic and contained a calcified spicule. Microscopically this mass was found to be a lymph node which had ruptured into, occluded and almost completely destroyed the bronchus (fig. 2*3*) at this level. Peripheral to this occluding mass, the bronchus (fig. 3*2*) was dilated and the seat of active tuberculosis. Adjacent to the dilated bronchus were three completely encapsulated caseous nodules (fig. 3*3*). The lymph nodes on the right were dense and anthracotic. The left superior bifurcation node contained a densely calcified nodule. No other areas of caseation or calcification were found in the lymph nodes.

This case was probably of the same type as the preceding one. The caseous nodule (fig. 2 *2*) was one of the lymph nodes of the primary complex and was in the line of lymphatic drainage from the primary focus to the hilus. The position of the lesion in juxtaposition to a medium-sized bronchus introduced an element more conducive to development of clinically manifest disease than to contiguous growth

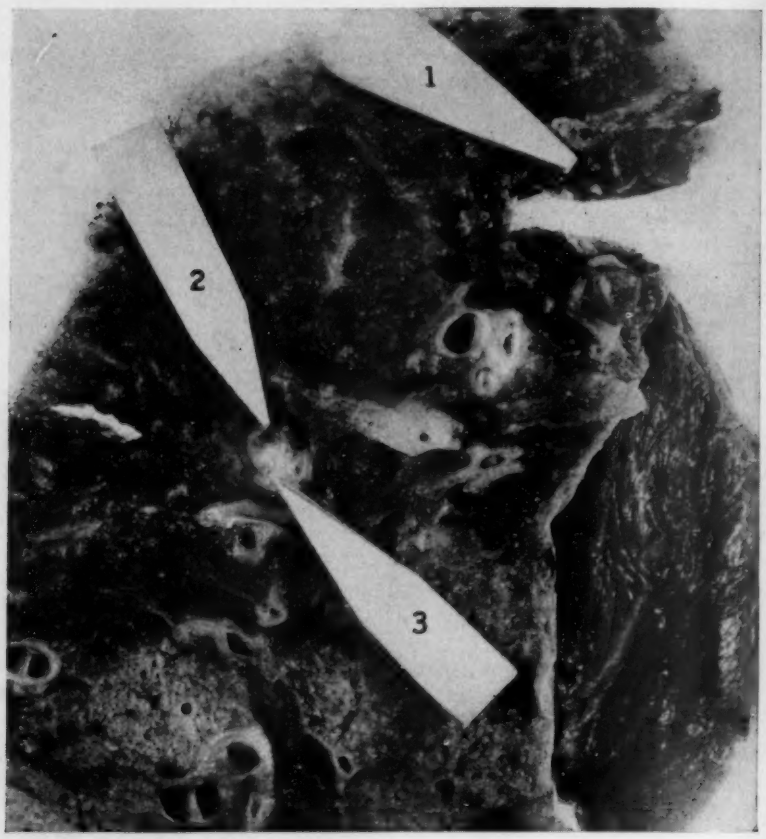

Fig. 2 (case 2).—*1*, primary focus; *2*, reactivated primary node; *3*, proximal portion of bronchus.

in the periphery of the lung as observed in the first case. The bronchial wall, already the seat of disease, must have shortly permitted generalized bronchiogenic distribution. The patient died before this event occurred.

Case 3.—An 18 year old white girl presented: tuberculous leptomeningitis; nontuberculous bronchopneumonia; obsolete tuberculous primary complexes of the

upper lobe of the left and lower lobe of the right lung and of the hilar lymph nodes, and active tuberculosis of the hilar nodes.

Beneath the pleura, in the mediastinal border of the upper lobe of the right lung was a small firm calcified nodule measuring 5 mm. in diameter. The dorsal pleura of the lower lobe of the left lung was retracted over a small calcified nodule lying subpleurally within the lung substance. This nodule was slightly larger than the other and measured 8 mm. in its greatest diameter. Microscopically both

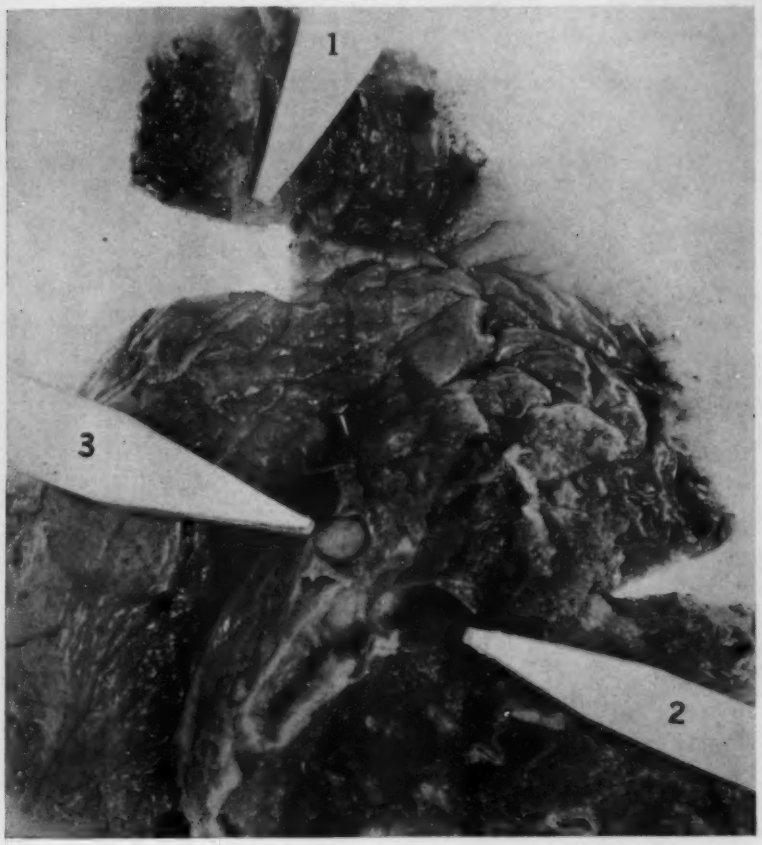

Fig. 3 (case 2).—*1,* primary focus; *2,* distal portion of a bronchus; *3,* peribronchial nodules.

nodules were calcified primary foci surrounded by thick fibrous capsules. The lung substance surrounding them was unchanged. A bronchopulmonary node lying anterior to the right main bronchus and beneath the pleural surface of the upper lobe measured 4 cm. in its largest diameter and was almost entirely replaced by extremely soft, slightly granular caseous material. The granularity of this material suggested the presence of calcium. In the superior bifurcation group, slightly to the right of the midline, there was another node, measuring 3 cm. in its greatest diameter, which was only partially calcified and which on section

showed caseous material, in which small firm granules were found. Several partially calcified smaller nodes were found, one along the inferior portion of the left main bronchus. Many microscopic sections of the nodes were examined. The right bronchopulmonary node showed amorphous masses that were partially calcified and surrounded by a dense anthracotic fibrous capsule. In this capsule were active tuberculous lesions. The left inferior bifurcation, left paratracheal and right paratracheal nodes showed the isolated obsolete tuberculous lesions characteristic of a primary infection, but no active lesions.

CASE 4.—A 39 year old Negress presented: chronic alcoholism; lymphogranuloma venereum (esthiomene); fatty metamorphosis of the liver (extreme);

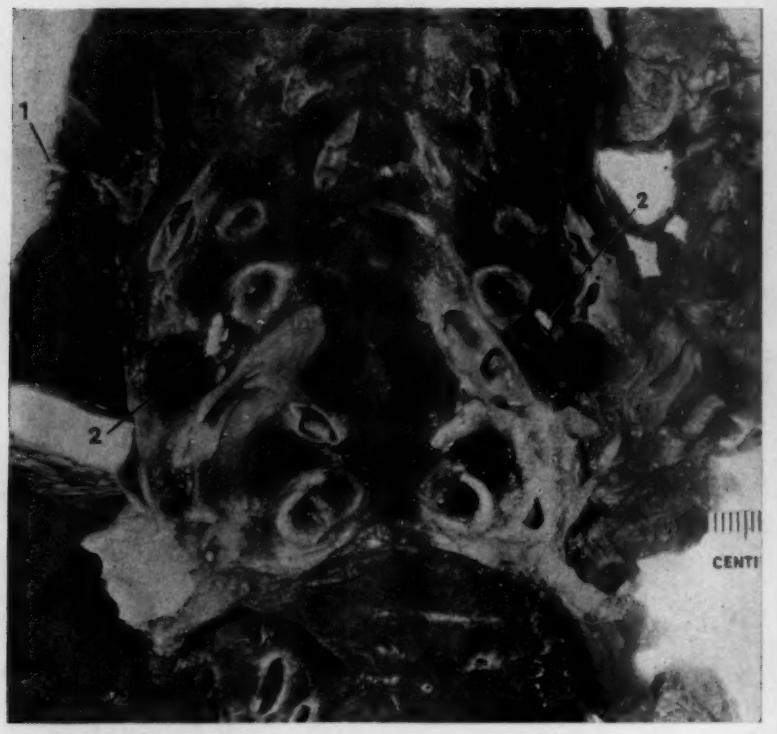

Fig. 4 (case 4).—*1*, retracted area of the pleura with a primary focus; *2*, calcified primary nodes.

acute nephrosis; arterial nephrosclerosis; chronic proctitis and periproctitis with partial obstruction and sinus formation; an obsolete primary tuberculous complex of the upper lobe of the right lung and hilar nodes, with fibrosis and bronchiectasis of the line of drainage; active conglomerate tuberculosis of the right upper bronchopulmonary node, and localized active conglomerate tuberculosis of the lower lobe of the right lung.

In the ventral surface of the upper lobe of the right lung, 6 cm. from the apex and beneath a pleural adhesion, was a retraction 2 cm. in depth (fig. 4 *1*). At the junction of the retracted lung and the minor interlobar fissure there was adhesion

between the visceral and the parietal pleura. Section of the lung through the retraction showed a stellate calcified scar extending toward the hilus. The small bronchi in the line of drainage were dilated. A bronchopulmonary lymph node below the eparterial bronchus contained a small petrified mass measuring 3 by 1 mm. (fig. 4 *2*). Other nodes in the line of drainage from the primary focus showed areas of massive caseation; the area in one node measured 6 by 10 mm. (fig. 5). The caseated areas bordered directly on the right main descending bronchus, on one hand, and on a medium-sized pulmonary vein, on the other. (Note arrows.) No gross invasion of the bronchial wall itself could be seen

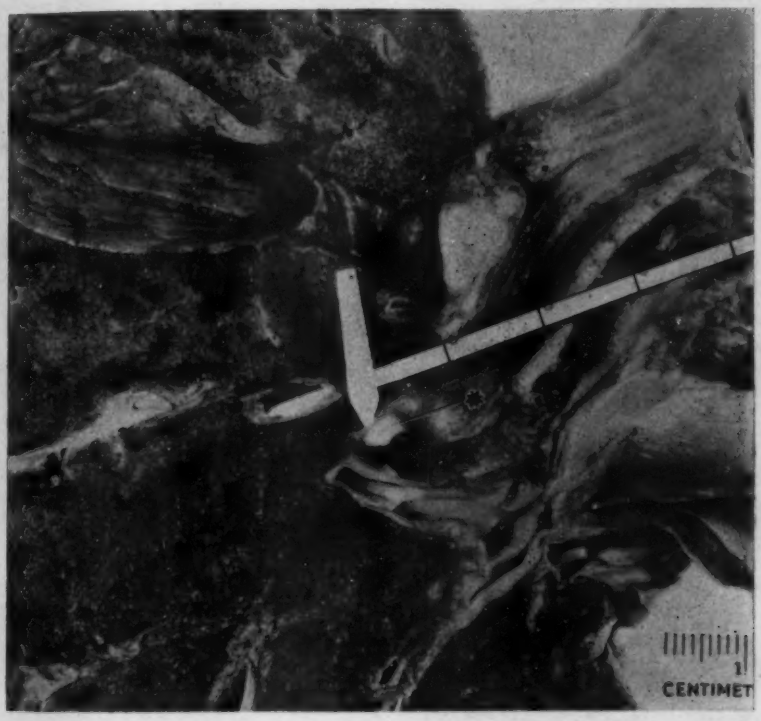

Fig. 5 (case 4).—The asterisk points to a reactivated primary node.

although the caseation reached directly to the bronchial cartilages. In the dorsal aspect of the upper portion of the lower lobe of the right lung was an area of conglomerate acinous-nodose tubercle formation measuring in its entirety 1 by 1 cm. (fig. 6). No other lesions resembling tuberculosis were found in the left lung. The right inferior bifurcation node contained petrified granules as well as apparently active tuberculous lesions.

Microscopic examination of the calcified lesion of the upper lobe of the right lung revealed a typical calcified primary focus with no evidence of activity. The lung between the focus and the hilus showed fibrosis of perivascular lymphatics and calcification of spaces—apparently lymphatics—in the walls of the dilated bronchi. There was no activity; however, the nodes at the hilus were the seat

of massive calcification and fresh caseating tuberculous lesions. One node showed a defect in the hyaline capsule of the area of calcification; the caseation of the surrounding active lesions extended through the defect into the calcified area itself (fig. 7). Another node adjoined the right lower bronchus. Here the tuberculous lesions had invaded the intercartilaginous spaces in the manner described in a previous article by Reichle and Frost.[3] Fresh tubercles were found in the submucosa. The lesion in the dorsal aspect of the lower lobe of the right lung was active conglomerate tuberculosis with perifocal tuberculous pneumonia.

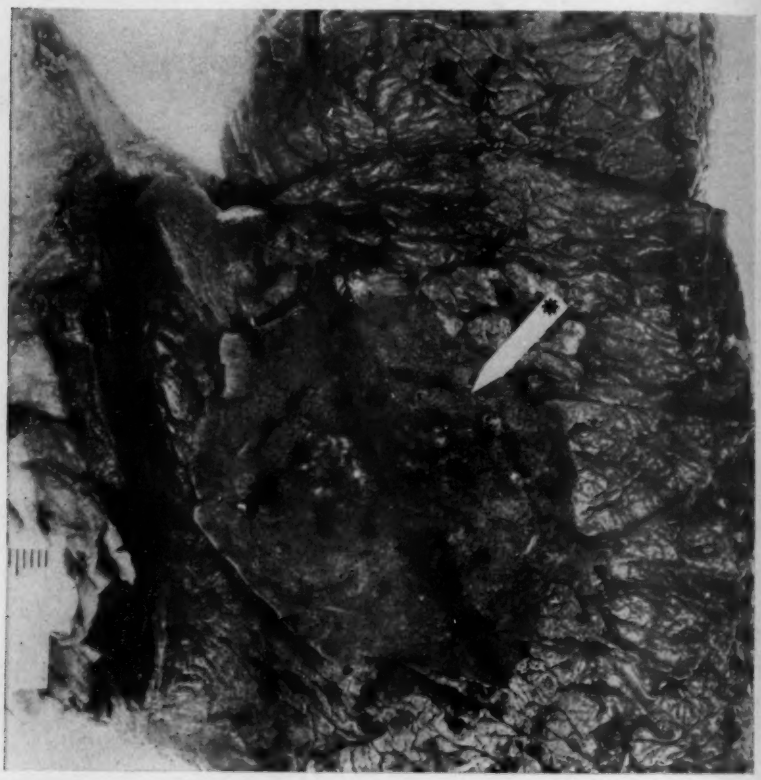

Fig. 6 (case 4).—The asterisk points to an area of bronchiogenic distribution.

CASE 5.—A 41 year old white man presented: active caseous tuberculosis of the right pleura and diaphragm, with empyema, and osteomyelitis of the ninth, tenth and eleventh ribs; tuberculous peritonitis; active tuberculosis of the right inferior bifurcation node with rupture into the bronchus; active conglomerate tuberculosis, bilateral (slight); miliary tubercles of the liver, spleen and kidneys (few and early), and obsolete primary tuberculosis of the right upper bronchopulmonary lymph node.

Between the visceral and the parietal pleura of the right lung there was a mass of yellowish white pasty material, which in some areas measured as much as

3. Reichle, H. S., and Frost, T. T.: Am. J. Path. **10**:651, 1934.

3 mm. in thickness. On section, well encapsulated caseous nodules were found distributed over the peripheral and interlobar pleura, but most frequently in the mediastino-diaphragmatic areas. These nodules measured up to 3.5 cm. in circumference and had dense fibrous capsules, which were firmly adherent to the wall of the chest. In a few areas of the apical region the lung was invaded to a slight degree by these caseous masses, but on the whole the parenchyma of the lung was not affected. There were a few well encapsulated small nodular conglomerations in the substance of the middle lobe and a very few isolated acinar-nodose lesions in the upper lobe. There was no cavitation.

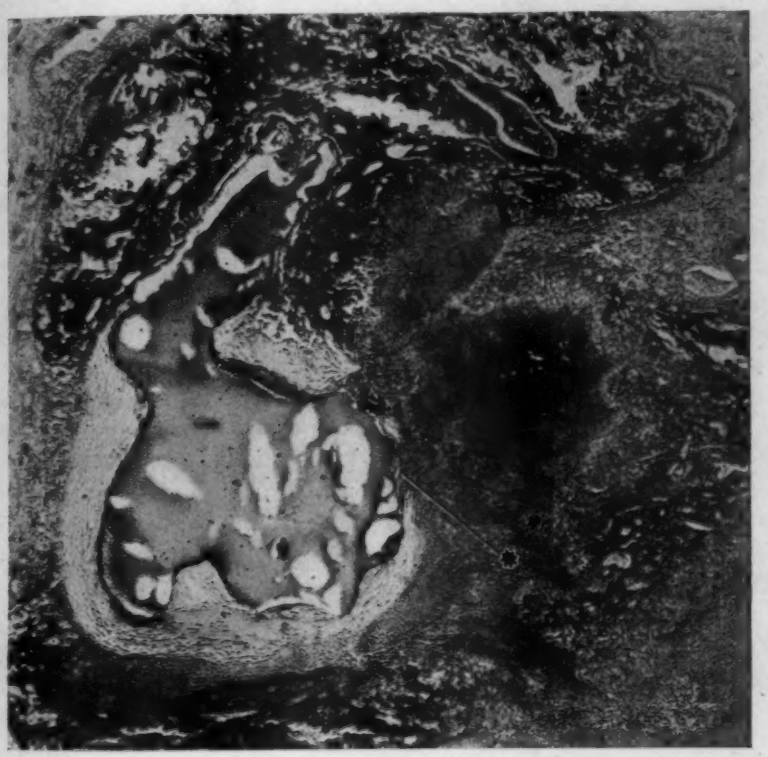

Fig. 7 (case 4).—The asterisk points to a defect in the fibrous capsule.

The pleura of the left lung was normal. On section a conglomerate acinar-nodose lesion measuring 1.3 cm. in its greatest diameter was found in the posterior axillary line of the upper lobe of the left lung. There was no peripheral reaction and very little fibrosis. Several smaller lesions of like character were found in the upper anterior axillary line and one in the subapical region.

The right inferior bifurcation lymph node measured 3 by 3 by 2 cm., was accompanied by marked periadenitis and on section was almost entirely caseous. The capsule of this node was absent in one area adjoining the lung. Here there was a communication between the lymph node and an adjacent mediastinal branch of the right secondary bronchus. The node also abutted on the pericardium, which at this point bulged and showed thinning and roughening. The right para-

tracheal nodes and the right bronchopulmonary nodes were small and anthracotic; in a right upper bronchopulmonary node measuring 8 mm. in diameter was a tiny calcified area. The lymph nodes on the left were small, dense and anthracotic.

In these three cases activity was shown in mediastinal nodes which were the seat of old primary tuberculous disease. In none of the cases were the primary tuberculous foci or their satellites active. In contradistinction to what was found in the first two cases, in these cases there was shown no evidence of distribution from the source of reactivation either to the lung itself or elsewhere. In case 5 the severe tuberculous pleurisy probably resulted from the disease in the bronchopulmonary node; the calcification of the adjacent node showed that this side of the lung was the seat of an old primary tuberculous infection. Since no other nodes in the chest were affected, it is unreasonable to suppose that the disease in this node was the result of drainage from tuberculous pleurisy. In addition to involving the serous surfaces, the lesion in the node had extended to the bronchus; early bronchiogenic distribution had already taken place at the time of the patient's death. In case 4 the disease of the bronchopulmonary node had given rise to bronchiogenic distribution; the lesions, however, were small and undoubtedly below the threshold of clinical observation.

Bronchiogenic distribution in this series of cases was the most frequent channel by which reactivation of a primary complex led to reinfection. Ghon's [1] mechanism of transmission by way of the lymph nodes to the lymphatic-venous angle and thence by way of the jugular vein to the general circulation could not be demonstrated in any of our cases. Case 3 may be an example of hematogenous distribution with the development of clinical disease. However, Ghon's mechanism cannot account for the site attacked, the meninges, since in such a case the infection would progress by the venous route and therefore, as in miliary tuberculosis subsequent to genital tuberculosis in the male, the lesions would be preponderantly pulmonary. If, therefore, case 3 represents hematogenous distribution from a reactivated pulmonary focus, the distribution must have occurred not by way of the lymphatic route but directly through the pulmonary vein to the major circulation.

In the foregoing investigation 700 routine autopsies were studied. Five cases thought to represent reactivation of primary foci were discovered incidentally. In addition, 100 routine autopsies were examined with the express purpose of determining the frequency of reactivation of the primary complex. Two cases were found. Neither of the series is statistically comparable with that of Anders.[4] Since it was not our purpose in the present study to establish the maximum number of cases of reactivation but rather to demonstrate the mechanism in well estab-

4. Anders, H. E.: Beitr. z. Klin. d. Tuberk. **72**:338, 1929.

lished instances, we excluded from consideration twenty-five cases of the second series in which there were active tuberculous foci not associated with primary infection and draining into the mediastinal lymph nodes. In these cases no investigation of the nodes was attempted.

CASE 6.—On a 57 year old woman thyroidectomy was done for thyrotoxicosis. Autopsy disclosed severe hemorrhages at the site of operation; bronchopneumonia; an obsolete promary tuberculous complex involving the lower lobe of the left lung and the left bronchopulmonary and inferior bifurcation lymph nodes.

The primary focus measured 6.5 mm. in diameter, was sharply circumscribed and had a capsule measuring 1 mm. The surrounding lung showed no tuberculous lesions. The primary nodes, which measured from 8 mm. to 3 cm. in their greatest diameters, were anthracotic and contained well demarcated encapsulated yellow lesions measuring from 1 to 15 mm. and of putty-like consistency. The rest of the nodes of the chest and upper part of the abdomen were hyperplastic and anthracotic but showed no gross evidence of tuberculosis.

The microscopic examination revealed fresh tuberculosis, small round cell infiltration, epithelioid cell proliferation and the giant cells of Langhans in and around the capsules of the lesions in the primary nodes.

The case shows an evidently remote primary complex associated with fresh tuberculous lesions in the nodes. No other evidence of tuberculosis was discovered; the case therefore represents either a very early reactivation of an obsolete primary complex or possibly continued subthreshold activity of a complex far advanced in healing.

CASE 7.—A 64 year old white man presented: rheumatic heart disease, healed, with aortic stenosis and mitral insufficiency and stenosis; chronic passive hyperemia of organs; bronchopneumonia, and calcified right superior bifurcation and bronchopulmonary nodes.

A calcified area was found in each of the nodes mentioned. All the nodes were small, dense and anthracotic. No fresh lesions suggestive of tuberculosis were found in the chest or elsewhere in the body. A small pretracheal node showed areas which appeared in the gross to be composed of hyalinized connective tissue. However, microscopic examination revealed fresh tubercles in the periphery of a hyalinized and anthracotic center. The rest of the nodes showed no active disease.

It is probable that the isolated activity of the pretracheal node represented extension from the primary complex, since this node was in the line of drainage and there was no demonstrable active tuberculosis elsewhere in the body.

Since Ghon's first paper on lymphonodular reactivation of primary tuberculous infection, there have been a number of important publications. The subject has been reviewed by Jaffé [5] and Pagel.[6] Anders [4] found reactivation of the nodal part of the primary complex in 35 per cent of all persons over 45 years of age. In addition to the ascending

5. Jaffé, R. H.: Arch. Path. **18**:712, 1934.
6. Pagel, W.: Am. J. M. Sc. **189**:253, 1935.

(cephalad) form of Ghon, he observed a descending (caudal) form, in which the lymph nodes in the posterior mediastinum and pancreaticolienal region were affected. In 30 per cent of the cases of reactivation of nodes this was associated with disease of organs other than the lung; the latter organ was rarely involved by active disease. No reactivated primary focus was seen. Subsequently Anders [7] described tuberculous lesions in the subpleural lymph nodules in 18 per cent of all persons more than 45 years old. These lesions he regarded as results of hematogenous distribution through the pulmonary artery from the reactivated mediastinal nodes described. Schmöe [8] confirmed these observations. Ghon and Kudlich [9] published their observations on a case of lymphonodular reactivation in a patient 23 years of age. In 1930 Kudlich [10] described six cases of lymphonodular reactivation in a total of 136 autopsies on children suffering from tuberculosis.

Although the fundamental morphologic aspects of reactivated primary infection are relatively well established, the bacteriologic features are a subject of dispute. Earlier workers reported observing consistently positive results on inoculating material from fibrotic, calcified and ossified nodes into guinea-pigs. Thus, Lydia Rabinowitsch [11] reported that in five cases of inactive tuberculosis "completely calcified lymph nodes" infected guinea-pigs with tubercle bacilli. The ages of the tuberculous persons were: 27, 35, 30, 46 years and "a child." It is important to note that three of these nodes were from the mesentery.

Most of the earlier publications, however, are of little value because the authors failed to distinguish between the lesions of primary infection (childhood type) and those of reinfection (adult type). The publication of Königsfeld and Puhl [12] is the first of critical value. In five cases the primary focus, and in eighteen cases the entire primary complex (parenchymal focus and primarily involved lymph nodes), was injected into guinea-pigs. According to the authors, the lesions were "obsolete." Two animals died of intercurrent infections; seven showed tuberculosis, and fourteen did not. The ages of the persons in whose cases the results were positive were: 41, 56, 64, 81, 67, 59 years and "an old man."

The report of Opie and Aronson [13] took issue with all previous opinions. It distinguished between childhood and adult lesions and, furthermore, between isolated childhood lesions and those associated

7. Anders, H. E.: Centralbl. f. allg. Path. u. path. Anat. **43**:25, 1928.
8. Schmöe, F.: Beitr. z. Klin. d. Tuberk. **71**:449, 1929.
9. Ghon, A., and Kudlich, H.: Med. Klin. **25**:54, 1929.
10. Kudlich, H.: Beitr. z. Klin. d. Tuberk. **75**:575, 1930.
11. Rabinowitsch, Lydia: Berl. klin. Wchnschr. **44**:35, 1907.
12. Königsfeld, H., and Puhl, H.: Ztschr. f. d. ges. exper. Med. **35**:340, 1923.
13. Opie, E. L., and Aronson, J. D.: Arch. Path. **4**:1, 1927.

with adult disease, remote or recent. In the latter group they obtained 4.4 per cent positive results from the inoculation of "caseous calcified" nodes, 20 per cent from "calcified," 33 per cent from "caseous fibrotic" and 23 per cent from "caseous encapsulated" nodes. The results for analogous lesions in the lung parenchyma were approximately the same. Opie and Aronson, however, discount the value of these rather high percentages because those lesions of childhood infection not associated with lesions of adult infection (isolated childhood type) showed only 8.1 per cent positive results from inoculations. This observation is not substantiated by the work of Anders,[14] who, with B. Lange, inoculated material from the lesions in a hundred cases. His classification and the results are as follows: I—thirty-two cases of isolated primary pulmonary complex, showing six positive results; II—seven cases of isolated mesenteric complex, showing no positive result; III—three cases of double pulmonary and mesenteric primary complex unassociated with reinfection lesions, giving two positive results; IV—nine cases of pulmonary primary complex associated with apical reinfection scars giving no positive result; V—four cases of intestinal primary complex and apical reinfection scars, giving no positive result. In all these cases the patients were over 45 years of age.

On the other hand, Schräder [15] in 1928 obtained only one positive result in 101 inoculations of calcific deposits from Ghon foci and primarily involved nodes. He included no case in which the primary complex was found associated with lesions of the reinfection type. It is difficult to harmonize these results with those of Anders and of Königsfeld and Puhl or even with those of Opie and Aronson, since the last obtained 8.1 per cent positive results from inoculations in a group similar to those of Schräder. Opie and Aronson suggested that the positive results obtained with the "obsolete" primary complexes were due to contamination with bacilli released from older reinfections elsewhere in the lungs. The fact that Rabinowitsch obtained positive results with mesenteric nodes and the fact that Anders found no tubercle bacilli in his groups IV and V but did find them in groups I and III are at variance with this suggestion. It is not advisable to assume, as did Opie and Aronson, that tubercle bacilli may lodge in living tissue without causing any reaction. This is not in accord with the generally accepted views concerning the relations between living host tissue and an invading organism against which the former is sensitized. The evidence presented by Opie and Aronson is open to the criticism that the grossly uninvolved lung tissue and nodes might have contained microscopic lesions since the block of tissue used measured 2 by 5 cm. and

14. Anders, H. E.: Beitr. z. Klin. d. Tuberk. **81**:260, 1932.

15. Schräder, G.: Virchows Arch. f. path. Anat. **269**:355, 1928.

the nodes 1 cm. This, of course, could not have been adequately examined and also used for inoculation.

In conclusion, therefore, it must be stated that the entire problem of the bacteriology of the primary complex requires further study on the basis of the facts learned from previous work and on a much larger and more heterogeneous group, in order to escape the errors of random sampling. The results hitherto obtained cannot outweigh the morphologic evidence presented here, which favors the assumption that there are viable organisms in an appreciable number of so-called obsolete primary complexes.

SUMMARY

Seven cases were observed in a total of 800 routine autopsies which yielded suggestive evidence of the existence of reactivated primary tuberculous infection. The frequency of the phenomenon cannot be determined by morphologic study, since the fugitive nature of reactivation, the fact that it must proceed to dissemination or subside in healing, tends to obliterate, in either case, the evidence necessary for its demonstration at autopsy. Since, despite these inherent difficulties, it is possible to demonstrate seven cases in 800 routine autopsies, endogenous reinfection should be accepted as a significant mode of extension of pulmonary tuberculosis.

EFFECT OF STAPHYLOCOCCUS TOXIN ON THE KNEE JOINTS OF RABBITS

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NASHVILLE TENN.

Rabbits and dogs inoculated intravenously with staphylococcus toxin and dying during the following twelve to twenty-four hours have focal hemorrhages in practically every organ. Animals surviving the lethal action of this toxin for a longer period frequently have hemorrhagic and necrotic lesions in their kidneys and in the mucosa of their gastro-intestinal tracts.[1] The injection of staphylococcus toxin into the colon of the dog produces local necrosis and ulceration.[2] Acute conjunctivitis and necrosis of the skin occur after local injection of a toxic staphylococcus filtrate into these tissues.[3]

Brunschwig and Jung[4] in 1931 apparently were the first to study the effect of staphylococcus toxin on joints. They injected a toxic filtrate into the knee joints of rabbits at twenty-four hour intervals for a period of from one to twenty-one days. Aseptic purulent arthritis developed. The lesion following these intra-articular injections of toxin was identical with the arthritis produced by staphylococci.

The present paper deals with observations made following a single intra-articular injection of staphylococcus toxin into the knee joints of rabbits. Each animal was studied clinically; roentgenograms were made at frequent intervals, and a pathologic study of the knee and of the adjacent soft tissues was made when the animal was put to death.

METHODS AND MATERIAL

Thirteen normal adult rabbits were used. The toxin was prepared by growing a hemolytic strain of Staphylococcus aureus in veal infusion broth.[5] A 0.02 cc. volume of this toxin produces complete hemolysis of 2 cc. of a 1 per cent concentration of rabbit's red blood cells suspended in physiologic solution of sodium chloride. A rabbit receiving intracutaneously 0.1 cc. of this filtrate shows after twenty-four hours an area of skin necrosis measuring 2 by 2 cm. The toxin has a p_H of 7.3.

From the Department of Pathology, Vanderbilt University Medical School.

1. Rigdon, R. H.: Arch. Path. **20**:201, 1935.
2. Rigdon, R. H.: Arch. Path. **23**:634, 1937.
3. de Christmas, M. J.: Ann. Inst. Pasteur **2**:469, 1888. Parker, Julia T.: J. Exper. Med. **40**:761, 1924.
4. Brunschwig, A., and Jung, A.: Rev. de chir., Paris **50**:521, 1931.
5. Rigdon, R. H.: Arch. Path. **22**:763, 1936.

The hair was shaved from the anterior surface of the knees, the skin was cleansed with iodine and alcohol, and 0.1 cc. of the toxin was injected through the midportion of the patella into the right knee joint. A 26-gage needle was used.

The rabbits were examined each day for the first ten days and at frequent intervals thereafter until they were put to death. Calipers were used to measure the distance between the condyles of the femur.

Roentgenograms were made of both knees at the intervals given in the table. The date on which the rabbits were put to death is also given in this table.

Tissues for histologic study were taken from the capsule of the knee and the adjacent soft tissue. The distal end of the femur was also removed. This material was fixed in a 4 per cent solution of formaldehyde. The bones were decalcified in a 5 per cent solution of nitric acid. All the sections were stained with hematoxylin and eosin.

Summary of Experiments

The right knee was inoculated with 0.1 cc. of staphylococcus toxin; the left knee, with 0.1 cc. of infusion broth. Roentgenograms of both knees were made on the experimental days given in the table. The date when the rabbits were put to death is also given.

Rabbit	Experimental Day—Roentgenograms of Knees Made	Days Until Put to Death
131	5, 19, 31, 41, 52, 66, 72	76
132	5, 11, 19, 31, 41	47
133	6, 14, 26, 36	42
134	6, 14, 26, 36, 47, 61	72
140	1, 9, 21, 31, 42, 56	67
141	1, 9, 21, 31	87
142	.....	3
143	.....	12
144	.....	Discarded 13th day
152*	23, 30, 44, 68	75
153*	.....	10
154*	44, 68	75
155*	.....	10

* These rabbits did not receive any infusion broth into the left knee joint.

Blood for culture was obtained from the heart at the time the animal was killed. The exudate in the right knee joint was cultured. All material for bacteriologic study was put into infusion broth. Smears of this exudate were stained by Gram's method.

The control for this experiment was as follows: A 0.1 cc. volume of infusion broth, p_H 7.5, was injected into the left knee joint of a rabbit which had been given the staphylococcus toxin in the right knee joint. The broth was injected at the same time and with the same technic as the toxin. Nine rabbits were used as controls.

CLINICAL OBSERVATIONS

The tissues about the right knee were swollen twenty-four hours after the joint had received the injection. The skin over both of the knees at this time was frequently hyperemic and slightly warmer than normal. The left knee and the adjacent tissues were usually normal forty-eight hours after the injection of the broth. The tissues around the right knee were markedly edematous and tender, and the skin was hyperemic for two days after receiving the toxic filtrate. The swelling

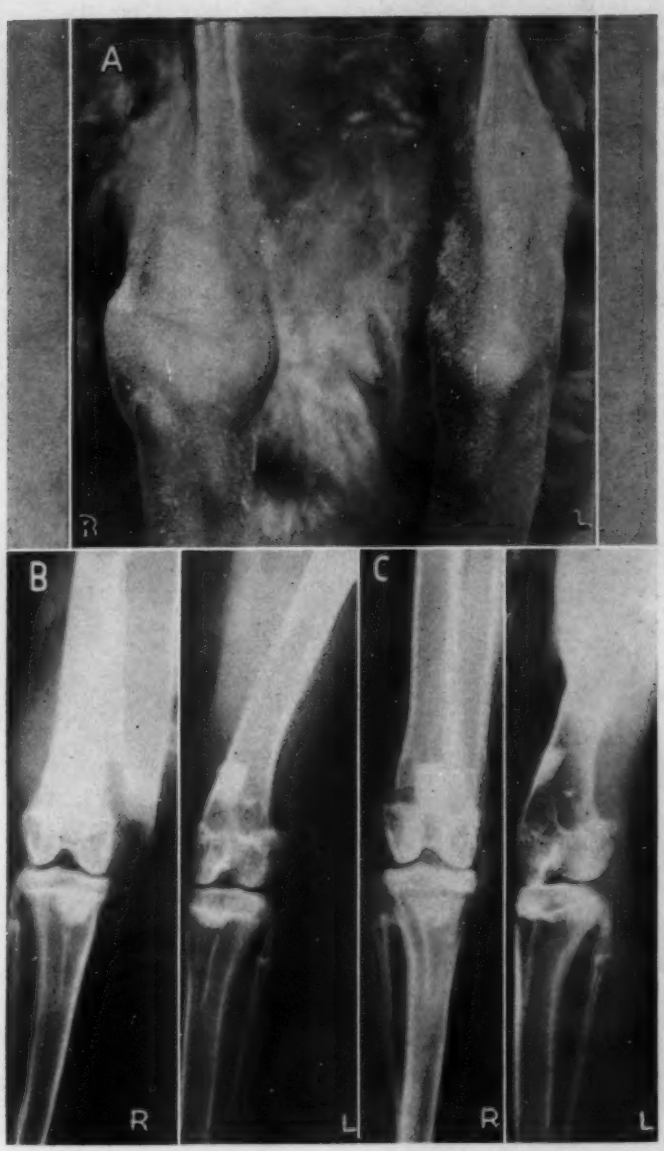

Fig. 1 (rabbit 131).—*A*, periarticular swelling about the right knee. The left knee is normal. The right knee received an intra-articular injection of 0.1 cc. of staphylococcus toxin fifty-five days previously. The left knee received an injection of 0.1 cc. of infusion broth at the same time. *B*, diffuse swelling in the soft tissues of the right leg eleven days after the inoculation of 0.1 cc. of staphylococcus toxin into the right knee. The left knee and leg are normal. *C*, swelling in the right leg, localized in the periarticular tissue about the knee, sixty-six days after the inoculation of the toxin. The left knee is normal.

of the soft tissues began to decrease gradually after the third day; however, it persisted for a longer time in the joint. The knee joints in the majority of the rabbits appeared normal by the twentieth postoperative day. The right knees of rabbits 131 and 152 were enlarged when these animals were killed on the seventy-fifth and seventy-sixth days of the experiment.

The swollen joints were moderately firm in consistency after the first few days. There were no fluctuant areas at any time. Pressure or movement of the right knee during the first ten days following the injection of toxin apparently produced severe pain. The right knee was held in flexion during this time, and any movement was avoided. A

Protocol of Rabbit 131

Experimental Days	Comment
4	Weight, 2,100 Gm. Right leg swollen and warm. Most of the swelling is in the muscles about the joint. Right leg held in flexion. Left leg and knee normal. Roentgenograms made.
5	Right knee held in flexion, unused.
6	Swelling in right leg decreasing.
7	Weight, 2,100 Gm.
11	Right knee and leg markedly swollen. Swelling extends down toward ankle. Roentgenograms made (fig. 1 B).
13	Weight, 1,925 Gm. Right knee swollen. Left knee normal. Roentgenograms made.
17	Right knee swollen and joint stiff.
19	Roentgenograms made.
21	Weight, 2,050 Gm.
22	Right knee 3 cm.; left knee 2 cm.
24	Right knee 3 cm.; left knee 2 cm.
26	Weight, 1,900 Gm. Right knee 2.9 cm.; left knee 1.9 cm.
31	Weight, 1,950. Right knee 3 cm.; left knee 2 cm. Swelling definitely localized in an area of the right knee joint. Roentgenograms made.
34	Weight, 1,900 Gm.
35	Weight, 1,950 Gm.
38	Weight, 2,000 Gm. Right knee 3 cm.; left knee 2.1 cm.
41	Roentgenograms made.
47	Weight, 1,950 Gm. Right knee 2.9 cm.; left knee 1.9 cm.
52	Weight, 1,900 Gm.
55	Photograph of knees made (fig. 1 A).
61	Weight, 1,800 Gm.
66	Right knee 2.6 cm.; left knee 2 cm. Roentgenograms made (fig. 1 C)
72	Weight, 1,750 Gm. Right knee 2.6 cm.; left knee 2 cm. Roentgenograms made.
76	Rabbit put to death.

return of function in the extremity usually accompanied a decrease in the swelling of the knee joint. Rabbit 152, however, had an enlarged knee on the seventy-fifth day of the experiment, although the function was normal.

The protocol of rabbit 131 shows the clinical observations made on one of the animals which received 0.1 cc. of staphylococcus toxin in the right knee and an equal quantity of infusion broth in the left.

ROENTGENOLOGIC EXAMINATIONS [6]

A periarticular swelling was present about the right knee of every rabbit except one (fig. 1 *B* and *C*). The left knee was normal. The

6. The roentgenologic interpretations were made by Dr. H. C. Francis of the department of roentgenology.

amount and duration of this periarticular swelling varied in the different animals. It usually appeared between the first and fourth day and began to decrease after from one to two weeks. Usually the periarticular swelling was completely absent after one month. The tissues about the right knees of rabbits 131 and 152, however, were swollen on the seventy-fifth and seventy-sixth days of the experiment.

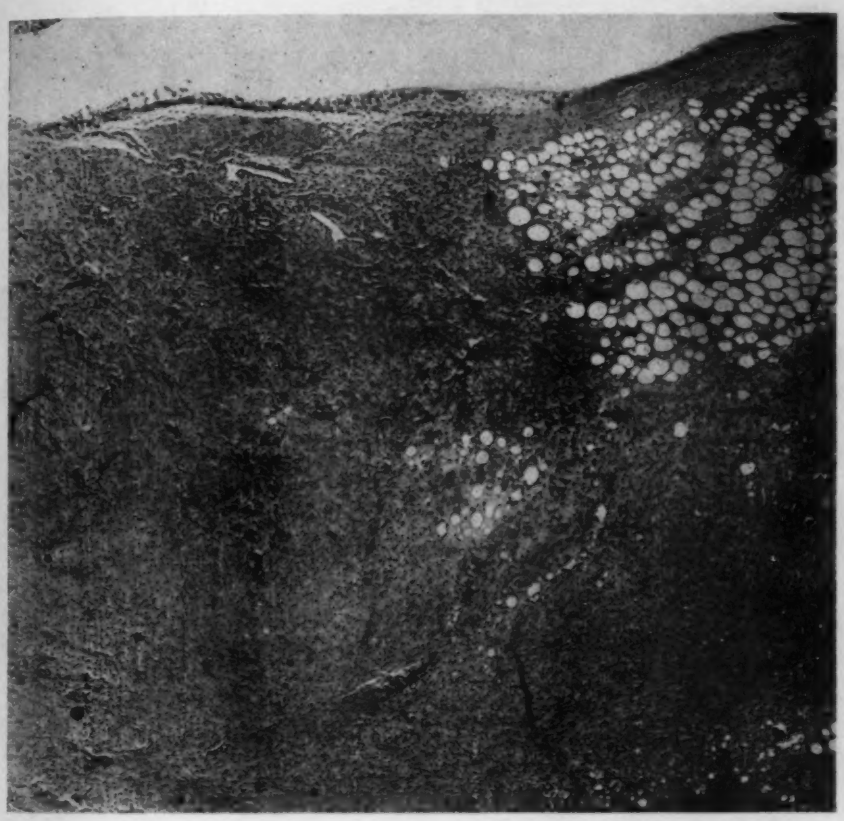

Fig. 2.—The capsule of the right knee of rabbit 152, thickened as a result of a proliferation of fibrous tissue. Few mononuclear cells are infiltrating the capsule. The joint cavity received an injection of 0.1 cc. of staphylococcus toxin seventy-five days previously.

The bones in the knee joints were normal.

BACTERIOLOGIC STUDY

Exudate from the right knee joints of rabbits 142, 153 and 154 was sterile after forty-eight hours' incubation. No bacteria were demon-

strated in the smears of the exudate from rabbit 153. Polymorphonuclear leukocytes and mononuclear cells were present in the smears.

Blood from the hearts of rabbits 131, 134, 140 and 153 was sterile after seventy-two hours' incubation.

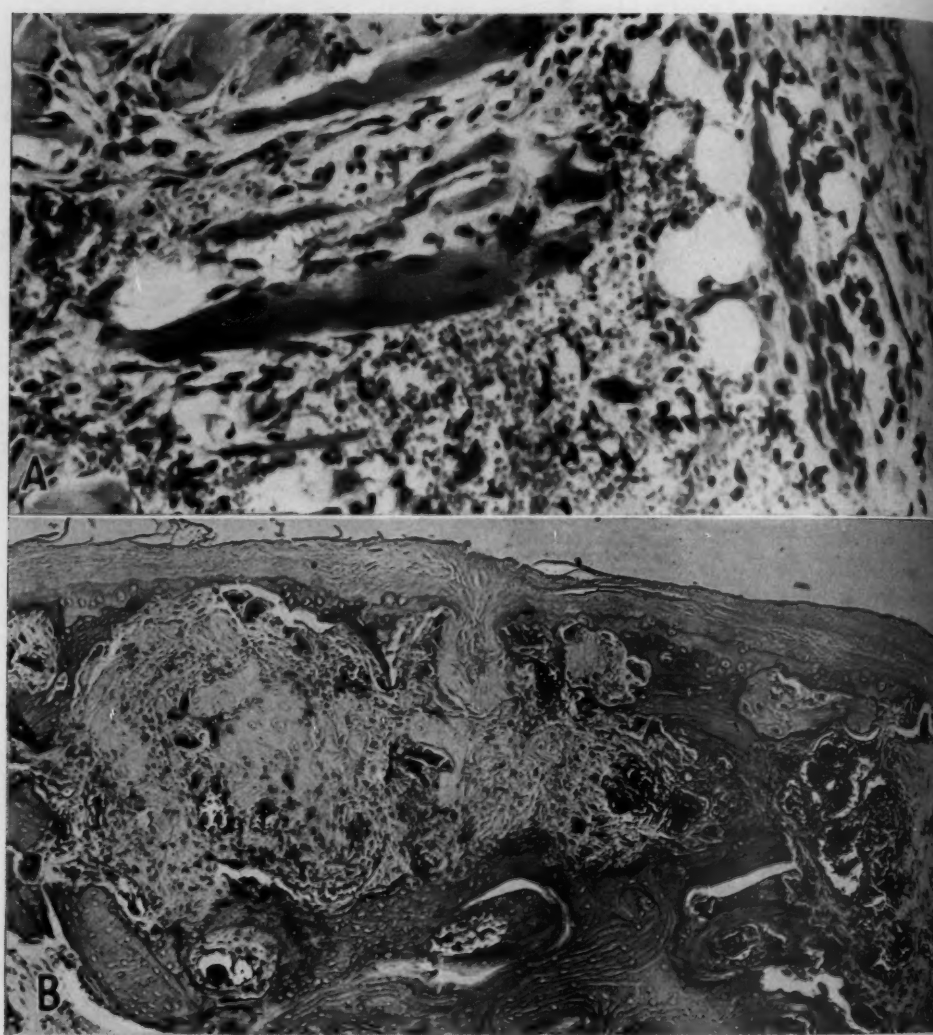

Fig. 3.—*A*, red blood cells and inflammatory cells infiltrating the periosteum and the soft tissues adjacent to the right knee of rabbit 153 ten days after 0.1 cc. of staphylococcus toxin was injected intra-articularly; × 250. *B*, epiphysis from the distal end of the femur of rabbit 131. The blood vessels have proliferated in a focal area of the epiphysis immediately below the articular cartilage. The cartilage apparently is being absorbed. The knee was given an injection of 0.1 cc. of staphylococcus toxin seventy-six days before the animal was put to death; × 95.

PATHOLOGIC STUDY

The soft tissues about the right knee were edematous and hemorrhagic seventy-two hours after the intra-articular injection of staphylococcus toxin into the joint. The muscles, soft tissues and capsule about the right knee were diffusely infiltrated with red blood cells, polymorphonuclear leukocytes and mononuclear cells (fig. 3*A*). The muscles were severely injured; some of the fibers were filled with vacuoles, and others were granular or hyaline. The cells lining the synovial membrane were swollen, and some were covered with a thin fibrinous exudate. The articular surface of the femur in some areas was also covered with a similar exudate. Few of the capillaries in the capsule and soft tissues were partially filled with fibrinous thrombi. The cartilaginous and bony tissues were normal.

The pathologic lesions on the tenth day were very similar to those observed on the third day. Fibroblasts, however, were beginning to appear in the capsule and in the adjacent soft tissues. The inflammatory reaction at this time was characterized by an increase in the number of mononuclear cells and a decrease in the polymorphonuclear leukocytes.

The tissues about the right knees of the majority of the rabbits that lived for fifty days or longer showed an increase of fibrous tissue and few mononuclear cells (fig. 2). An occasional small villus projected from the synovial membrane into the cavity. The cartilage was eroded from small areas on the articular surface of the femur in some of the rabbits. The areas in which the cartilage was absent were congested in the gross, and microscopically they showed proliferation of fibrous tissue at the base and increase in the number of small blood vessels (fig. 3*B*). Multinucleated cells, apparently osteoclasts, were present in these areas. No inflammatory reaction was present in the epiphysis or metaphysis of the femur.

No pathologic lesions were present in the capsule, soft tissues or femur in the left leg.

COMMENT

The results obtained in this experiment indicate that staphylococcus toxin when injected intra-articularly into the knees of normal adult rabbits produces injury to the articular cartilage, synovial membrane, capsule and adjacent soft tissues. The reaction is characterized by hemorrhage and by exudation of polymorphonuclear leukocytes and mononuclear cells. Proliferation of fibrous tissue follows the acute reaction.

The clinical, roentgenologic and pathologic observations are all coherent. It is difficult to determine either clinically or with roentgenograms the presence or absence of fluid in the joint cavities in the acute phase of this experiment. The rabbits that were killed before the

fifteenth day, however, showed only a small amount of thin blood-tinged fluid, which could not be confused with the exudate in purulent arthritis.

Focal areas in which the articular cartilage is absent suggest that staphylococcus toxin injures hyaline cartilage. Furthermore, it appears that these injured cells are removed by a process of absorption which begins at the inferior surface of the cartilaginous membrane. Such a process for the removal of injured cartilage has been described by Nichols and Richardson [7] and by Allison and Brooks.[8]

The apparent fixation of the right knees of rabbits 131 and 152 may be the result of proliferation of fibrous tissue about the capsule. No adhesions were found between the opposing articular surfaces.

The results obtained in this experiment following a single intra-articular injection of staphylococcus toxin differ from those of Brunschwig and Jung.[4] These investigators found an aseptic purulent arthritis after multiple inoculations of staphylococcus toxin into the knee joints of rabbits. The arthritis produced by Brunschwig and Jung was identical with the septic arthritis caused by the staphylococcus.

Frequent intra-articular injections of large quantities of many different types of substances will subsequently produce pathologic changes in and about joints. Such a study has been made by Key.[9] He produced chronic arthritis by the frequent intra-articular injection of from 1 to 2 cc. quantities of weak acids, weak alkalis or distilled water. The volume of the inoculum used in my experiments apparently was too small to cause any pathologic change either in the joint or in the adjacent tissues. The filtrate was also approximately neutral.

Pathologic lesions of the type observed in this experiment apparently are the results of the action of staphylococcus toxin on the cells in the articular cartilage, synovial membrane, capsule and tissues about the joint. This toxin apparently diffuses through the capsule or follows the tendinous portion of the muscles to reach the periarticular tissues.

CONCLUSION

Staphylococcus toxin when injected intra-articularly into the knee of the normal rabbit produces injury to the articular cartilage, synovial membrane, capsule and adjacent tissues. An acute and subsequently a chronic inflammatory reaction follows this injury.

A periarticular swelling as shown by roentgenograms follows the intra-articular injection of staphylococcus toxin into the knee joint of the normal adult rabbit. The process is primarily periarthritis.

7. Nichols, E. H., and Richardson, F. L.: J. M. Research **21**:149, 1909.

8. Allison, Nathaniel, and Brooks, Barney: Surg., Gynec. & Obst. **19**:568, 1914.

9. Key, J. A.: J. Bone & Joint Surg. **15**:67, 1933.

Case Reports

ARTERIOVENOUS HAMARTOMA OF THE BRAIN

Report of a Case with Autopsy

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Angioma of the brain is generally regarded as a congenital anomaly or a malformation of the vascular system, although with the reservation that it is capable of further alteration, both in size and in character. It is to be differentiated from the frequently encountered hemangioblastoma, which is generally considered to be a true neoplasm. The distinction becomes somewhat difficult at times because occasionally a hemangioblastoma of the cavernous type is encountered, but Cushing and Bailey [1] stated that the presence of intervascular gliotic tissue is evidence of the hamartomatous character of the angioma. The angioma is to be distinguished from the capillary telangiectasis, although occasionally it is difficult to determine just where the line is to be drawn between the congenital telangiectasis and the angioma. The term "telangiectasis" is preferably restricted to the small lesion mainly capillary in nature which, commonly observed in the skin, is also sometimes found in the brain.

Angioma of the brain, whether purely venous or arteriovenous (angioma arteriole), is an uncommon lesion, and although it has been reported occasionally, there have been relatively few pathologic studies. The case reported here is of interest not only because of the tissue available for microscopic study but because of the presence of a second lesion, a small meningeal fibroblastoma.

REPORT OF CASE

A white woman 48 years of age was admitted to the accident ward of Lakeside Hospital, in coma.

The history, obtained from a son, was limited. For the past twenty years the patient had suffered from seizures characterized by stiffness, rigidity and slight convulsive attacks but without loss of consciousness. There apparently had been no characteristic site of origin, but there had been a sufficient prodromal period to allow the patient to prepare for such a seizure. Urination and defecation occurred during and immediately following such attacks, and the patient had often complained of pains in the left side of the chest and back during the episodes. Tinnitus had occasionally been associated with the attacks.

In the past four years attacks had become infrequent, but during the past six months transient blurring of vision had been noticed during the seizures, and visual acuity had in general become much reduced. On the day before admission the patient experienced a severe attack, following which she vomited.

From the Institute of Pathology, Western Reserve University.

1. Cushing, H., and Bailey, P.: Tumors Arising from the Blood Vessels of the Brain, Springfield, Ill., Charles C. Thomas, Publisher, 1928.

She became comatose and was brought into the hospital twenty-five hours later without having regained consciousness.

Examination revealed a well developed obese white woman, unconscious and with frothy yellowish white sputum coming out of the mouth. The right pupil was larger than the left, but both reacted to light. Examination of the fundi revealed papilledema with engorgement of the veins and tortuosity of the arteries on the nasal side. The heart was not enlarged to percussion, and the cardiac sounds could not be heard. Blood pressure was 156 systolic and 70 diastolic. Scattered coarse râles and rhonchi were present throughout the lungs. The patellar reflex and Babinski response were absent on each side.

Lumbar puncture gave bloody spinal fluid under a pressure of 150 mm. of water. The patient died a few hours after admission without having regained consciousness. The clinical diagnosis was that of cerebral hemorrhage.

The autopsy was performed by Dr. F. B. Jeppesen. The positive pertinent observations were limited to the brain. The heart was flabby but not enlarged, and vascular tumorous lesions in organs other than the brain were not found. The spinal cord was not examined.

The cerebrospinal fluid covering the brain was blood-tinged, and all the venous radicles were markedly hyperemic. The cerebral convolutions showed some flattening, and there was a definite pressure cone of the cerebellar tonsils. Over the right temporal region there existed an area of softening which, when incised, revealed a large blot clot. A small amount of clotted blood was found in the cisterna magna and beneath the leptomeninges around the medulla and upper cervical part of the cord, apparently representing drainage from the hemorrhage into the ventricular system. The brain was fixed in solution of formaldehyde U. S. P. (1:10) and thoroughly hardened.

On section, the entire ventricular system was found to be filled with clotted blood and the septum pellucidum pushed over to the left of the midline. In the right hemisphere, a large subcortical cystic cavity, which measured roughly 9 by 6 cm., was situated between the cortex and the right lateral ventricle and, in its major portion, occupied the inferior parietal lobe. It extended downward into the temporal lobe and backward into the occipital lobe and, in this region, communicated with the posterior horn of the right lateral ventricle. The cavity was completely distended with clotted blood and was lined with necrotic brain tissue. The cavity apparently represented recent hemorrhage into an area of encephalomalacia.

Posteriorly and below the cystic area described was a depressed area of cortex which, from the external gross appearance, was interpreted as old hemorrhage. It was situated in the postero-inferior portion of the right temporal lobe and extended well back into the occipital lobe. On section, it was found to measure 5 by 3 by 2 cm. The lesion consisted of a wedge-shaped mass of tangled, somewhat thick-walled vessels which extended into the right lateral ventricle (fig. 1). Many of the vessels were apparently in close contact, while others were separated by cerebral tissue, which was the seat of hemorrhage. Calcification of many of the vessels was grossly evident. The overlying dura was not involved, although it appeared somewhat more vascular than usual. The vessels at the base of the brain were not markedly sclerotic, and none was calcified. No unusually large vascular channels, either supplying or draining the lesion, were noted.

Attached to the dura in the left middle cranial fossa, and projecting into the brain, although not attached to it, was a small gray round mass, somewhat

flattened, which measured 1.5 by 0.5 cm. It was soft and elastic and presented a granular cut surface.

Microscopic Examination.—Sections from various parts of the brain were stained by the hematoxylin-eosin, Van Gieson trinitrophenol solution-fuchsin, Mallory phosphotungstic acid-hematoxylin and Weigert resorcinol-fuchsin methods and by the Verhoeff method for elastic tissue, both with and without the Masson trichrome counterstain. Several blocks of tissue required decalcification in Steve's reagent prior to embedding. The following description is limited to the two tumorous lesions in the brain.

The vascular lesion was composed of a tangle of interlacing blood vessels which varied in diameter from 1 mm. or less to more than 5 mm. Occasional

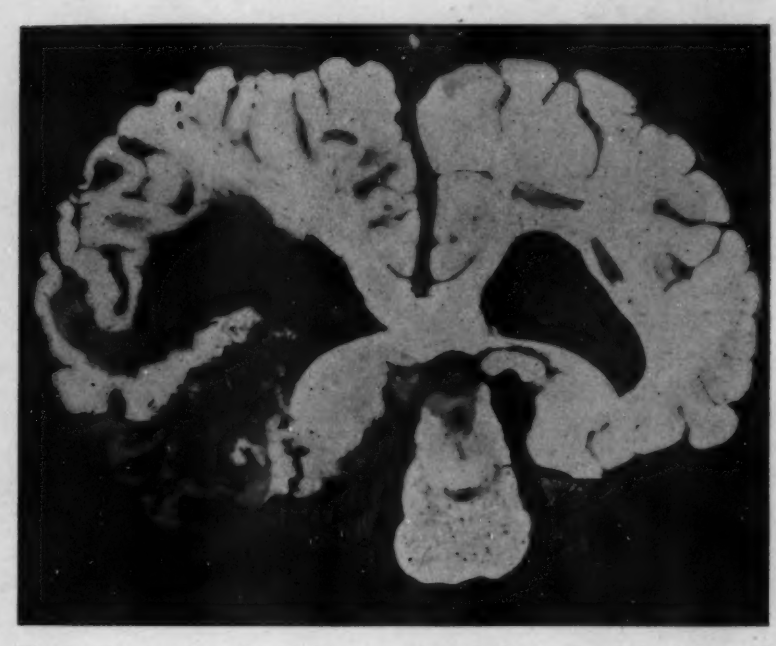

Fig. 1.—A coronal section of the brain through the center of the lesion. Note the wedge-shaped mass of tangled vessels which extends into the right lateral ventricle.

vessels were in contact, but most of them were separated by a variable amount of cerebral tissue, which was the seat of an inconstant degree of gliosis. In the main, the intervening tissue showed a remarkable degree of preservation despite the extent and nature of the lesion. In many places moderately well preserved ganglion cells were present. A few lymphocytes and plasma cells were scattered throughout the tissue in the region adjoining the lesion, although this was not a conspicuous feature. Most of the blood vessels contained erythrocytes, although a few were empty. Several of the vessels were completely sclerotic and converted into hyalinized fibrous cords.

The vessels were for the most part arteries, although veins were also present. Typically, they were thick-walled, but there were few in which the wall was of uniform thickness. The intima usually consisted of a single row of flattened,

elongated cells, but there were present numerous moderately thick flat ingrowths or nodules which arose from the intima and projected into the lumens of the vessels (fig. 2). These plaques contained many elastic and collagenous connective tissue fibers and occasionally tended to split the lamina elastic interna into

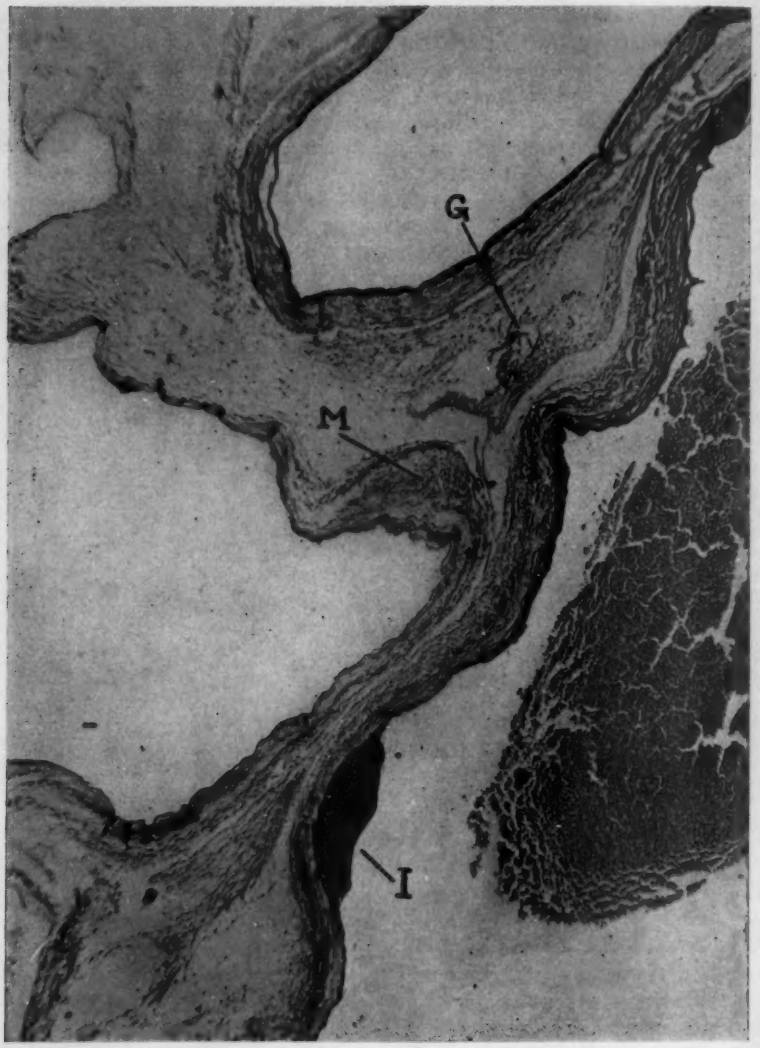

Fig. 2.—A cross-section of the vascular lesion. Note the intimal proliferation (*I*), the focal overgrowth of the media (*M*) and the intervening partially degenerated gliotic tissue (*G*). (Verhoeff's elastic tissue stain, × 46; yellow filter.)

several indistinct layers. The latter structure in many instances was well developed, but occasional vessels were seen in which the internal elastic layer

was but partially present. In these, although one part of the wall was decidedly arterial, another portion was seen to consist merely of a single layer of endothelium, a few scattered elastic fibers and a small amount of collagenous connective tissue. Occasional reduplication or splitting of the internal elastic layer was seen where the intima was thickened.

A distinct media was present in most of the vessels, and in some places an external longitudinal and an internal circular layer of smooth muscle could be distinguished. Here again, however, there was a marked tendency toward variation in the thickness of this coat. An occasional focal thickening was seen which sometimes was purely muscular or occasionally contained an admixture of connective tissue. The media was further the seat of varying degrees of fibrosis, and in a few places coarse granules of calcium salts were present. Large plaques of calcific deposits were fairly numerous, but these were never sufficiently large to occlude the lumen completely. The lamina elastica externa was, in general, poorly developed throughout and in most instances consisted of scattered short fragmented elastic fibers, while an occasional vessel was seen in which it was entirely lacking.

The adventitia also varied in thickness but was for the most part relatively thin. Aside from foci of hyalinization, it was not remarkable. Capillaries in the immediate region of the lesion were in the main moderately, although occasionally enormously, dilated. In the walls of a few of these smaller vessels granules of calcium salts were also found.

The lesion involved the overlying leptomeninges, from which it probably arose. It differed here in that it appeared less extensive and less severe. The lesion was regarded as an arteriovenous hamartoma (angioma) of the cerebrum, with secondary and terminal rupture into the ventricular system.

Sections taken through the small tumor mass found projecting into the brain in the left middle cranial fossa showed it to be adherent to, and intimately related with, the dura, although the demarcation between the tumor and the dura was distinct. There was a scanty capsule composed of a few strands of connective tissue, although this was a most inconspicuous feature of the growth. Neither brain nor dura was invaded by the growth. The tumor was composed of sheets of cells of the connective tissue type. These contained fair-sized oval or round, highly chromophilic nuclei and a moderate amount of cytoplasm, which was bipolar in disposition. The stroma of the tumor was scanty and supported numerous small thin-walled vessels. About the vessels the tumor tissue tended to be somewhat more cellular and formed whorls in which the relationship between the tumor and the walls of the vessels was intimate. Mitotic figures were infrequent. The picture was that characteristic of the meningeal fibroblastoma.

COMMENT

It is unfortunate that the nature of the case precluded any extensive clinical survey which might have given some insight into the actual character of the lesion before death supervened. The case lacked practically all of the clinical signs and symptoms which lead to a diagnosis of an intracranial vascular lesion of this nature. The only clinical signs which could in any way be associated with this lesion were the engorgment of the retinal veins and the extreme tortuosity of the retinal arteries, epileptiform attacks which appeared to simulate those of the jacksonian type, tinnitus during such attacks and lastly the prolonged clinical course. It is emphasized that these were in no way diagnostic or even suggestive of the nature of the lesion

in this case but that in reconstructing the picture they appear to fit in with the observations at autopsy.

Cushing and Bailey [1] have shown that the typical aneurysmal angioma may fail to give the full-blown syndrome of audible intracranial bruit, increased extracranial vascularity, unilateral nonpulsating exophthalmos, choked disks and epileptiform attacks.

Pathologically, lesions of the type described are of interest from the point of view of both their origin and the changes which they undergo. Wolf and Brock [2] in a pathologic study of nine cases of angioma of the brain, two of which were of the arteriovenous type, suggested certain clinical and microscopic features which point to enlargement of such lesions. Other writers [3] were similarly led to conclude that these lesions probably enlarge. Much of the evidence to this effect is, of course, indirect. As an example, there is no way of knowing whether in certain cases the lesion is arteriovenous to start with or whether, primarily venous, it subsequently becomes partially arteriolized. From the relatively few pathologic studies available, it appears that both conditions may exist, although it is at times impossible in a single given case to determine microscopically the exact status of the lesion in question.

Histologically, the case reported here was interpreted as one of an arteriovenous hamartoma, the term "hamartoma" being used in preference to "angioma" for reasons to be discussed subsequently. The various microscopic features of the lesion differed in no important way from those recorded by other writers. The added presence of a meningeal fibroblastoma is an unusual feature which has been described but rarely.

The meningeal fibroblastoma found in this case was purely an incidental finding at autopsy and was probably in no way related to the clinical picture. Although in the past, multiple primary intracranial tumors have been reported from time to time, the literature contains little in respect to the association of intracranial tumors with vascular tumorous malformations. Sands [4] reported a case in which there was in one frontal lobe both an endothelioma and an angioma, but he gave no description of the vascular lesion. The vascular lesions reported by Ohlmacher [5] have been regarded as telangiectases. In this case there was, at autopsy, aside from other changes referable to the central nervous system, a meningioma. Hosoi [6] reported a case in which multiple meningiomas were associated with numerous angiomatous nodules of the right frontal lobe. Both from the description and from the photomicrographs it seems probable that the lesions here represent telangiectases rather than true angiomas.

It must be admitted that there exists much confusion regarding the nomenclature of blood vessel tumors and tumorous formations of the central nervous system. Much of this no doubt arises from the fact that in many instances it has been impossible to determine whether or

2. Wolf, A., and Brock, S.: Bull. Neurol. Inst. New York **4**:144, 1935.
3. Dandy, W. E.: Arch. Surg. **17**:190, 1928. Cushing and Bailey.[1]
4. Sands, I. J.: J. Nerv. & Ment. Dis. **62**:157, 1925.
5. Ohlmacher, A. P.: J. Nerv. & Ment. Dis. **26**:395, 1899.
6. Hosoi, K.: Am. J. Path. **6**:235, 1930.

not a given lesion has a hamartomatous basis. The fact that a hamartomatous lesion may secondarily become blastomatous, coupled with the fact that many of the vascular lesions are prone to be organoid, makes the determination doubly difficult. Only too often in the past a nonspecific, indefinite term has been employed which through common usage has subsequently come to imply an entirely different meaning from that which was originally intended. The use of such ill-chosen terms in attempts at classification has probably contributed much toward the diversity of opinion in respect to the allocation of tumors of various types to their related groups.

The term "angioma" has come to mean a tumor composed of newly formed blood vessels,[7] although in the past it has been applied more or less indiscriminately to vascular blastomatous and hamartomatous lesions alike. The term is best restricted to those lesions which are undoubtedly blastomatous, and as such it should not be applied to either hamartomatous vascular malformations or telangiectases. The latter lesions are not uncommon, and although they may occasionally appear tumorous, as in the case described by Hosoi,[6] they are not neoplastic. Telangiectases may be acquired in the central nervous system, but the majority are congenital and may even have a familial basis.[8]

The term "hamartoma" has been used here in preference to "angioma" since the lesion described was unequivocally on a congenital basis. The pathogenesis of such vascular malformations is as yet uncertain. In a recent monograph on the subject, Bergstrand, Olivecrona and Tönnis[9] reviewed the literature and offered a somewhat different classification than has been accepted hitherto by writers in this country.

The presence of a primary intracranial neoplasm with one or more vascular malformations is probably more than fortuitous. The interpretation in the case given here appears to be similar to that given by Hosoi,[6] namely, the suggestion of a congenital or dysontogenetic susceptibility of the mesodermal germ layer.

SUMMARY

A case of arteriovenous malformation (angioma) of the right cerebral hemisphere associated with a small meningeal fibroblastoma of the left middle cranial fossa is reported with the gross and microscopic observations at autopsy.

Emphasis has been placed on the manner in which characteristic arteriovenous malformations may fail to give clinical signs characteristic of this type of lesion.

The hamartomatous nature of the vascular lesion has been emphasized, and the terminology of the group of blood vessel tumors has been briefly touched on.

7. Ewing, J.: Neoplastic Diseases, Philadelphia, W. B. Saunders Company, 1928, p. 240.

8. Kufs, H.: Ztschr. f. d. ges. Neurol. u. Psychiat **113**:651, 1928. Michael, J. C., and Levin, P. M.: Arch. Neurol. & Psychiat. **36**:514, 1936.

9. Bergstrand, H.; Olivecrona, H., and Tönnis, W.: Gefässmissbildungen und Gefässgeswülste des Gehirns, Leipzig, Georg Thieme, 1936.

Laboratory Methods and Technical Notes

A CHROMIUM-HEMATOXYLIN STAIN FOR MYELIN IN PARAFFIN SECTIONS

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The best methods for staining myelin sheaths of nerve fibers with hematoxylin are based on treatment of the pieces of tissue with a chromium compound *before dehydration and embedding.* The chemical basis of this procedure is generally believed to be as follows: The chromium salts combine with the lipids of the myelin sheath to form compounds insoluble in the dehydrating and clearing fluids that are used preparatory to embedding in celloidin or paraffin. The chromium-lipid compound, according to this interpretation, is present in the finished sections and is avid of hematoxylin, hence the blue-black staining of myelin sheaths. A full discussion of this matter will be found in Bertrand.[1]

The prechroming[2] procedures are slow and cumbersome. The penetration of chromium salts into the specimens of nerve tissue is slow. The moment when the penetration is complete is difficult to estimate. Yet, if the chroming bath is terminated too soon, the deep portions will stain more lightly than the periphery and may give the impression of demyelinization. Furthermore, the chromed blocks are difficult to cut and yield brittle sections. Aside from the myelin stain, other technics, such as the Nissl methods, cannot be used on chromed specimens.

For all these reasons, many authors have attempted hematoxylin stains for myelin without prechroming. Most of these methods are variants of Heidenhain's ferric hematoxylin lake. The most successful is Loyez' method,[3] which can be applied to both pyroxylin (celloidin) and paraffin sections from pieces fixed in solution of formaldehyde U. S. P. The stain, while often excellent, is sometimes uneven and often somewhat inelective, glia fibers, fibrin, elastic fibers and collagen all being stained to some extent. Here it is evident that the action of the ferric mordant cannot be that of rendering myelin lipids insoluble, since during dehydration and embedding they have already been dissolved out as much as they ever will be. The action of the iron can be

From the Department of Pathology of the Central and Neurological Hospital, Department of Hospitals.

1. Bertrand, Ivan: Techniques histologiques de neuropathologie, Paris, Masson & Cie, 1930.

2. The terms "prechrome" and "postchrome," used in this paper, refer to the treatment of tissue specimens with a chromium compound before and after dehydration and embedding.

3. Loyez: Compt. rend. Soc. de biol. **69**:511, 1910.

only that of electively combining with the insoluble residue and producing a hematoxylin lake, exactly as in staining of chromatin and centrioles. This suggests an alternative to the usual explanation of the mechanism of prechroming. May not the chromium compound act on the insoluble fraction simply as a mordant for the production of a chromium-hematoxylin lake?

If this hypothesis is correct, the difference in results between the prechroming and the ferric methods is due chiefly to the more elective mordanting of myelin by the chromium compound, and this difference should persist in postchromed pyroxylin (celloidin) or paraffin sections.

In an effort to test this hypothesis and also to seek a more satisfactory stain for myelin in dehydrated material, the following chromium-hematoxylin lake method was developed:

1. Fixate in solution of formaldehyde U. S. P.
2. Carry paraffin sections to water.
3. Place sections in Weigert's primary mordant for twelve hours.
4. Rinse.
5. Stain with Kultschizki's hematoxylin for one hour at 56 C.
6. Rinse.
7. Place sections in a 1 per cent lithium carbonate solution for a few seconds, to turn blue.
8. Wash at the tap for one hour.
9. Place in a 0.3 per cent potassium permanganate solution for a few seconds.
10. Rinse.
11. Differentiate carefully in Pal's solution.
12. Wash at the tap for at least one hour.
13. Dehydrate, clear and mount in balsam.

Weigert's primary mordant is made as follows:

Potassium bichromate	5 Gm.
Fluorchrome	2 Gm.
Distilled water	100 cc.

Kultschizki's hematoxylin is made as follows:

Hematoxylin	1.5 Gm.
Absolute alcohol	15 cc.
Glacial acetic acid	2 cc.
Distilled water	100 cc.

Pal's solution is made as follows:

Oxalic acid, 1 per cent solution Sodium sulfite, 1 per cent solution	equal amounts of each

Following step 6, the technic is identical with the Weigert-Pal-Kultschizki method as employed by Miss Rosa Bay-Ramyon in the laboratory of the Neurological Hospital. Substitution of 2 per cent potassium dichromate solution in step 3 and of Weigert's borax-ferricyanide differentiator for Pal's method gave a less satisfactory stain.

The result is a constant and even myelin stain, the electivity of which is distinctly superior to that of Loyez' ferric lake and is comparable to that of the Weigert-Pal method with prechroming. In

particular, glia fibers, collagen and all other interstitial structures are a uniform transparent pale yellow or light brown, against which the intense blue-black of myelin sheaths stands out strongly. Nuclei are generally stained more deeply than in prechromed specimens. Hemoglobin and heme bodies [4] stain brown, as in prechromed specimens.[5]

This method has been used for over eight months on a variety of material. Together with Trelles' [6] method of neurofibril impregnation, which is currently employed on paraffin sections, the postchroming method for myelin has contributed a material saving of time and effort in the neuropathologic technic as practiced in this laboratory. The success of postchroming speaks in favor of a purely mordant-like action of chromium compounds on an insoluble myelin residue. To determine the nature of this chromaffin myelin fraction is a task for the chemist.

4. Liber, A. F.: Arch. Path. **21**:863, 1936; J. Nerv. & Ment. Dis. **85**:286, 1937.

5. Liber, A. F., and Lisa, J. R.: Rosenthal Fibers (Heme Bodies) in Non-Neoplastic Syringomyelia, J. Nerv. & Ment. Dis., to be published.

6. Trelles, J. O.: Rev. neurol. **1**:459, 1932.

General Review

STAPHYLOCOCCIC IMMUNITY

RÉSUMÉ OF EXPERIMENTAL AND CLINICAL STUDIES

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NASHVILLE, TENN.

Ogston [1] in 1883 gave the name "staphylococcus" to one of the two types of organisms which he frequently found in suppurative lesions. It was his opinion that a ptomaine produced by this organism was the injurious agent in certain types of inflammation. An alcoholic extract was prepared by Leber [2] from cultures of staphylococci. De Christmas [3] obtained a filtrate from a culture of a staphylococcus in veal bouillon which produced edema and suppuration when injected into the anterior chamber of the rabbit's eye. Between 1883 and 1888 these three substances were thought to be associated with staphylococci; each produced an inflammatory reaction.

During the following quarter of a century considerable information about the filtrate was obtained. Van de Velde [4] prepared staphylococcus filtrates from blood, serum and bouillon mediums and demonstrated in vitro their toxic action on leukocytes. He gave the name "leukocidin" to that toxic fraction of the filtrate which destroyed leukocytes. The observation was made by van de Velde [4] and Kraus and Clairmont [5] that red blood cells were hemolyzed by the toxic filtrate. Necrosis was produced in the skin of rabbits by injecting the same material locally.[6] The term "skin-necrotizing factor" has been given to the fraction in the filtrate that produces this dermal lesion. Necrosis was also observed in the kidneys of rabbits which had been given staphylococcus filtrates intravenously.[7] "Nephrotoxin" was the term given to the fraction which

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1. Ogston, A.: J. Anat. & Physiol. **16**:526, 1881; **17**:24, 1882.
2. Leber, M.: Fortschr. d. Med. **6**:460, 1888.
3. de Christmas, M. J.: Ann. Inst. Pasteur **2**:469, 1888.
4. van de Velde, H.: Cellule **10**:403, 1894.
5. Kraus, R., and Clairmont, P.: Wien. klin. Wchnschr., 1900, p. 49.
6. von Lingelsheim, W.: Aetiologie und Therapie der Staphylokokken-Infectionen, Berlin, Urban & Schwarzenberg, 1900.
7. Neisser, M., and Levaditi, C.: Compt. rend. Cong. internat. de méd. (sect. de path. gén.), 1900, p. 475.

produced this renal lesion. Rabbits died in from five to thirty minutes after the intravenous injection of staphylococcus filtrates.[8] The "acute killing factor" was the name applied to the fraction which caused this sudden death.

There is some doubt as to whether or not the different reactions produced by toxic staphylococcus filtrates are the results of the action of one or more fractions.[9] At present no one has proved this point satisfactorily; however, it appears to me that staphylococcus poison contains a single toxic substance which acts on different tissues in similar ways.

Staphylococcus toxin may be obtained by growing selected strains of staphylococci in veal infusion broth for forty-eight hours in the presence of a small amount of agar, carbon dioxide and diatomic oxygen. The broth filtrates have been shown to contain a hemolysin, leukocidin, skin-necrotizing factor, lethal factor, nephrotoxin and gastro-intestinal toxin. The toxin apparently is an exotoxin, appears early in the filtrate, is heat labile, slowly oxidizable and antigenic, can be converted into a toxoid by formaldehyde and is neutralized by staphylococcus antitoxin.

The antihemolysin in immune serums is readily demonstrated. A technic for determining the presence and quantity of this antibody in serum was developed by Neisser and Wechsberg [9a] and by Parish and his co-workers.[10] The standard unit of staphylococcus antitoxin is based on the hemolytic, skin-necrotizing and lethal factors in the toxin.[11]

Dr. G. W. McCoy,[12] director of the National Institute of Health, stated that

> The hemolytic unit of a toxin (H. U.) is the smallest amount of toxin which causes complete hemolysis (4+) of 1 cc. of 1 per cent saline suspension of fresh washed rabbit red blood cells.
>
> The hemolytic test dose (L. H.) is that quantity of staphylococcus toxin which is just neutralized by 1 unit of United States standard antitoxin,[12a] as is evidenced by inhibition of hemolysis.

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8. Kraus, R., and Pribam, E.: Wien. klin. Wchnschr. **19**:493, 1906.

9. (*a*) Neisser, M., and Wechsberg, F.: Ztschr. f. Hyg. u. Infectionskr. **36**:299, 1901. (*b*) Nélis, P.: Ann. Inst. Pasteur **52**:597, 1934.

10. Parish, H. J.; O'Meara, R. A. Q., and Clark, W. H. M.: Lancet **1**:1054 1934.

11. Hartley, P., and Smith, M. L.: Quart. Bull. Health Organ., League of Nations, 1935, p. 68.

12. Personal communication to the author, Sept. 2, 1936.

12a. "One unit is contained in 0.0009502 Gm. of the United States standard staphylococcus antitoxin." This definition is quoted from: U. S. Standard Staphylococcus Antitoxin, Bulletin B-1199, United States Treasury Department, National Institute of Health, January 1936.

The dermonecrotic dose (R. D.) is the smallest amount of toxin which causes necrosis in an area 0.5 cm. in diameter when injected intradermally [13] into the skin of a healthy albino rabbit.

The dermonecrotic test dose (L. R.) is that quantity of toxin which is just neutralized by 1 unit of United States standard antitoxin, as manifest by inhibition of dermonecrosis.

The minimum lethal dose (M. L. D.) is the smallest amount of toxin which causes the death of a healthy white mouse weighing between 17 and 22 Gm.

The lethal test dose (L +) is that quantity of toxin which is just neutralized by 1 unit of United States standard staphylococcus antitoxin, as manifest in the survival of the test animal (white mice).

It is evident from the foregoing definition that a unit of staphylococcus toxin is the converse of a unit of staphylococcus antitoxin, for which an arbitrary international standard has been adopted.

"Staphylococcus antitoxin" and "staphylococcus antihemolysin" are terms often used interchangeably in the literature. This mistake in usage makes the exact interpretation of the various articles in which results of investigations have been reported difficult. This error in terminology is present in this paper where the results of these investigations are discussed.

The amount of antihemolysin in serum is often considered an index to the host's capacity for resisting staphylococcic infection. Dolman [14] stated that patients treated with staphylococcus toxin show clinical improvement when the antihemolytic titer is increased. Blair and Hallman [15] in following the antihemolytic titer failed to observe any clinical improvement in a series of patients similarly treated. Antihemolysins may be an accurate index to the host's capacity for resisting staphylococcic infection. It appears to me, however, that this problem deserves further investigation.

IMMUNITY FOLLOWING RECURRENT STAPHYLOCOCCIC INFECTIONS AND VACCINE THERAPY

The treatment of recurrent staphylococcic infections of the skin is a difficult problem. It appears that persons with this type of infection should have immunity of a higher degree than the "normal," since the intact micro-organism is antigenic. One must remember, however, that the production of immunity is not dependent on the antigen alone but also on the reactive capacity of the host.

13. Here "subdermally" has been changed to "intradermally." Personal communication, April 15, 1937, from Dr. W. G. Workman, acting chief, Division of Biologics Control, National Institute of Health.

14. Dolman, C. E.: Lancet **1**:306, 1935.

15. Blair, J. E., and Hallman, F. A.: Proc. Soc. Exper. Biol. & Med. **34**:637, 1936.

The antihemolytic titer of the serum in cases of sycosis and other superficial staphylococcic infections is slightly above the normal.[16] Dolman,[17] however, has found that the antihemolytic titer of the serums from patients with recurrent furunculosis does not differ from the normal.

According to some experimental results, animals repeatedly given intradermal injections of cultures of Staphylococcus aureus acquire partial immunity.[18] Other investigators (Ramon and others,[19] Nélis [20] and Gengou [21]), however, have found that rabbits given staphylococcus vaccines do not show any increased resistance to a lethal dose of virulent staphylococci. Murray [22] stated that as far as evidence has been collected, the injection of dead staphylococci does not appear to cause any increase in the antitoxic titer.

Downie [23] recently studied the relative importance of the antibacterial and antitoxic factors in immunity to experimental infections in the rabbit. He used either heat-killed suspensions of staphylococci or formaldehyde-treated filtrates from cultures of the same strain. The antihemolytic titer of the serums was greatly increased when toxoid or toxin was used as the antigen, while essentially no change occurred in the antihemolytic titer following immunization with the vaccine. Rabbits which had been immunized with vaccines showed lesions in the skin similar to those in normal animals, whereas the lesions in the rabbits immunized with culture filtrates were much less extensive and showed a more active phagocytic response on the part of the tissue cells.

There is diversity of opinion on the clinical value of vaccines in the treatment of staphylococcic infections. Barber and Forman [24] felt that their results with the intradermal injection of vaccines in cases of sycosis were very encouraging. Klotz and Holman [25] stated that the use of vaccines with killed cocci has a long and variable history of great successes and failures.

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16. Bryce, L. M., and Burnet, F. M.: J. Path. & Bact. **35**:183, 1932. Conner, J. I., and McKie, M.: ibid. **37**:353, 1933.

17. Dolman, C. E.: J. A. M. A. **100**:1007, 1933.

18. Panton, P. N., and Valentine, F. C. O.: Brit. J. Exper. Path. **10**:259, 1929.

19. (*a*) Ramon, G.; Djourichitch, M., and Richou, R.: Compt. rend. Soc. de biol. **122**:1160, 1936. (*b*) Ramon, G.; Richou, R., and Djourichitch, M.: Rev. d'immunol. **5**:482, 1936.

20. Nélis, P.: Rev. d'immunol. **1**:152, 1935.

21. Gengou, O.: Ann. Inst. Pasteur **48**:135, 1932.

22. Murray, G. S.: Lancet **1**:303, 1935.

23. Downie, A. W.: J. Path. & Bact. **44**:572, 1937.

24. Barber, H. W., and Forman, L.: Brit. J. Dermat. **45**:4, 1935.

25. Klotz, O., and Holman, W. L.: Am. J. M. Sc. **189**:436, 1935.

IMMUNITY PRODUCED BY STAPHYLOCOCCUS TOXOID

Owing to the uncertainty of therapeutic success with vaccines attention has been diverted to staphylococcus toxoid as an antigen.

Burnet [26] in 1929 apparently was the first to detoxify staphylococcus filtrates with formaldehyde. Two rabbits immunized by intradermal injections of this formaldehydized toxin were found resistant to the skin-necrotizing and acute killing action of the unformaldehydized filtrate. The amount of antitoxin in the serum of both man and rabbit can be increased by intradermal, subcutaneous and intravenous injections of toxoid.[27] The experiments of Conner and McKie [27a] indicated that active immunization with toxoid protects animals against staphylococcic infections. Their immunized rabbits survived the intravenous injection of many lethal doses of either toxin or cultures of staphylococci. In a few instances the animals were alive after two months. Many investigators have been of the opinion that circulating antitoxin is of value in infections produced by virulent staphylococci.[28]

Clinical studies have been made on the efficiency of staphylococcus toxoid in the treatment of staphylococcic infections.[29] Conner and McKie [27a] chose to study the effect of staphylococcus toxoid in cases of sycosis, since there is usually very little tendency to spontaneous cure of this type of cutaneous infection. Complete healing occurred in ten of the eighteen cases in which treatment with toxoid was given. At the time of their report, in four of the cases the infection had reached a stage in which only a few pustules appeared each week. In four other cases the patients were still under treatment.

Whitby [29a] stated that in 100 cases of localized staphylococcic infection staphylococcus toxoid gave more consistent results in treatment than any other antigen. A rise in the antihemolytic titer of the serum was present in all these cases. In relatively few of the cases did the condition fail to respond clinically to this treatment. He emphasized the fact that patients vary in their response to the toxoid. Some respond to small doses; others require continuous treatment. Whitby attempted to raise the antihemolytic titer to at least ten times that for the original level rather than to name a fixed unit of antihemolysin above which any patient may be said to be immune.

Ramon and his collaborators [19a] and Dolman [14] apparently have treated the largest number of patients with staphylococcus toxoid. The

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26. Burnet, F. M.: J. Path. & Bact. **32**:717, 1929.

27. (*a*) Conner, J. I., and McKie, M.: Brit. J. Dermat. **46**:20, 1934. (*b*) Parish and others.[10]

28. Ramon and others.[19b] Nélis.[20] Gengou.[21] Conner and McKie.[27a]

29. (*a*) Whitby, L. E. H.: Lancet **2**:779, 1934; (*b*) **1**:1454, 1936. (*c*) Nélis, P.: Presse méd. **43**:1141, 1935. (*d*) Parish and others.[10] (*e*) Dolman.[14] (*f*) Ramon and others.[19b] (*g*) Murray.[22] (*h*) Conner and McKie.[27a]

conditions which they have treated include recurrent furunculosis, osteomyelitis, staphylococcic infections of the nose, throat and accessory nasal sinuses, pyelonephritis, blepharitis and pyopneumothorax. In general, favorable results appeared to follow the use of toxoid. Others have failed to obtain good results when staphylococcic infections of the skin were treated with toxoid.[30] Kindel and Costello [30a] treated forty-two patients, twenty-eight of whom had acne vulgaris, six furunculosis and eight sycosis vulgaris. Following the treatment with toxoid eight of the patients showed a slightly improved and thirty-four an unimproved or worse condition. Furuncles developed in two of the patients after large doses of toxoid had been given.

Cornbleet and Rattner [30b] used toxoid to treat approximately fifty patients who had staphylococcic infections. Their results with this form of therapy were similar to those of Kindel and Costello.

IMMUNITY PRODUCED BY STAPHYLOCOCCUS TOXIN

A third form of staphylococcic immunity is that produced by staphylococcus toxin. This antigen offers an excellent opportunity for experimental study, but it is not satisfactory for clinical purposes.

The antitoxic or antihemolytic titer of serums can be increased by a series of intravenous or intradermal injections of a staphylococcus filtrate.[31] Rigdon [32] produced, by a series of intraperitoneal injections in rats, complete protection against the lethal dose of staphylococcus toxin. Stookey and his co-workers [33] actively immunized rabbits with staphylococcus toxin and found them protected against several times the lethal dose of toxin. Erlsbacker and Saxl [34] failed to obtain antitoxin in the serum of rabbits after a three weeks' course of subcutaneous injections of staphylococcus toxin. It is of interest to know that the toxin used by Erlsbacker and Saxl produced no injury when injected subcutaneously into normal rabbits. Parker [35] injected toxic filtrates intravenously into rabbits and observed little, if any, production of antitoxin.

Greenbaum and Harkins [36] treated a small group of patients who had chronic staphylococcic pyoderma with a staphylococcus filtrate and

30. (*a*) Kindel, G. T., and Costello, M. J.: J. A. M. A. **102**:1287, 1934. (*b*) Cornbleet, T., and Rattner, H.: ibid. **102**:1780, 1934.

31. Murray.[22] Burnet.[26]

32. Rigdon, R. H.: J. Lab. & Clin. Med., to be published.

33. Stookey, P. F.; Scarpellino, L. A., and Weaver, J. B.: Arch. Surg. **32**: 494, 1936.

34. Erlsbacker, O., and Saxl, P.: Wien. klin. Wchnschr. **45**:39, 1932.

35. Parker, J. T.: J. Exper. Med. **40**:761, 1924.

36. Greenbaum, S. C., and Harkins, M. J.: J. A. M. A. **90**:1699, 1928.

obtained some clinical improvement. Weise's [37] results were also favorable in a small group of patients with staphyloderma who were treated by subcutaneous injection of a toxic staphylococcus filtrate. The number of cases is too small, however, for the results to be significant.

IMMUNITY PRODUCED BY STAPHYLOCOCCUS ANTITOXIN

Another method of immunization became possible when the observation was made that the antitoxic titer of serum was increased following a series of injections of either staphylococcus toxin or toxoid. This antitoxin neutralizes the toxin in vivo and in vitro.

The intravenous injection of this antitoxin completely protects animals against the lethal action of staphylococcus filtrates.[8] It also protects rabbits and guinea-pigs against the toxin derived from several strains of staphylococci. Parker and Banzhof [38] in 1926 apparently were the first to attempt to produce staphylococcus antitoxin in the horse. Their experiment was successful, and today staphylococcus antitoxin is prepared commercially. This antitoxin will completely protect rabbits against a lethal dose of staphylococcus toxin and neutralize the so-called hemolytic and skin-necrotizing factors either in vivo or in vitro.[39]

The effect of antitoxin on rabbits inoculated with cultures of staphylococci has been studied. Burnet [26] gave a group of rabbits a lethal dose of a broth culture of staphylococci and immediately thereafter gave them varying amounts of antitoxin. Usually the rabbits receiving the bacteria in the afternoon were dead by the following morning, while those which received the immune serum died during the second night. Stookey and his associates [33] observed that rabbits given antitoxin lived longer following the intravenous injection of virulent staphylococci than did the controls.

Parish, O'Meara and Clark [10] found that rabbits which had had varying amounts of serum intravenously immediately before the intravenous injection of virulent cultures lived from four to thirty days. A group of six rabbits "which had been injected with antitoxin intravenously twenty-four hours before the virulent culture in the same experiment showed even better protection, for five were still alive and well after one month." The control animals for this group "died in 6 to 16 hours." "It has been rare in our experiments to get protection as complete as in this experiment."

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37. Weise, E. C.: J. A. M. A. **95**:324, 1930.

38. Parker, J. T., and Banzhof, E. T.: J. Immunol. **13**:25, 1927.

39. (*a*) Joyner, A. L., and Smith, D. T.: Surg., Gynec. & Obst. **63**:1, 1936. (*b*) Dolman, C. E.: Canad. M. A. J. **30**:601, 1934. (*c*) Ramon, G.; Bocage, A.; Richou, R., and Mercier, P.: Presse méd. **44**:281, 1936.

Staphylococcus antitoxin has been used in the treatment of staphylococcic infections in man.[40] The general opinion is that this antitoxin has a definite therapeutic value in the treatment of certain staphylococcic infections. Gross [41] emphasized the fact that the use of antitoxin is of prime importance in staphylococcic septicemia as well as in severe osteomyelitis, furunculosis and carbuncles of the face and neck, when symptoms of toxemia are marked.

A recent statement has been made by Stookey and his associates,[32] as follows:

> We have to date studied the toxin-forming properties of twenty-seven strains of staphylococci obtained from cases of osteomyelitis. It is interesting to note that in twenty-nine cases of osteomyelitis the staphylococcus was isolated twenty-seven times. Of the twenty-seven cultures, twenty-four were hemolytic, twenty-two necrotic, three nonhemolytic and five non-necrotic. If one considers hemolysis as evidence of the formation of toxin, this would mean that in twenty-seven cases of staphylococcic osteomyelitis twenty-four of the invading organisms were toxin makers.

CONGENITAL TRANSFER OF STAPHYLOCOCCIC IMMUNITY

Relatively few observations have been made on the transfer of staphylococcic immunity from mother to offspring. Very young rats born from naturally immune mothers have been found to possess antitoxin, which disappears later in life.[16] Rigdon [32] showed that 30 day old rats born from mothers that were immunized survived a lethal dose of toxin. Furthermore, in this study on passive immunity it was suggested that antibodies may reach the young by passing through the placenta. One injection of toxin given during the period of gestation is usually insufficient to produce complete immunity in the young. This amount of antigen is also insufficient to protect the mother against the lethal dose of staphylococcus toxin. The greatest degree of protection for the young is obtained by giving several injections of toxin during the period of gestation. When the mothers are immunized during the period of lactation the young rats are not immune to the lethal dose of the toxin.

The probabilities are that either the antigen passes through the rat's placenta to the fetus and there stimulates the formation of antibodies or the maternal antibodies pass through the placenta to the young. No one has shown conclusively the mechanism of this transferred immunity.

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40. (*a*) Panton, P. V.; Valentine, F. C. O., and Dix, V. W.: Lancet **2**:1180, 1931. (*b*) Parish, H. J., and Clark, W. H. M.: J. Path. & Bact. **35**:251, 1932. (*c*) Dolman, C. E.: Canad. M. A. J. **31**:1, 1934. (*d*) Ramon and others.[19a] (*e*) Ramon and others.[19b] (*f*) Joyner and Smith.[39a] (*g*) Dolman.[39b]

41. Gross, H.: Ztschr. f. Immunitätsforsch. u. exper. Therap. **73**:14, 1931.

ANTIBACTERIAL IMMUNITY

Topley [42] said that "our knowledge of the exact method by which bacteria are killed in the tissues is woefully incomplete." Observations have been made which show that bacteria are phagocytosed by polymorphonuclear leukocytes and cells of the reticulo-endothelial system. Either normal or antibacterial serum aids this process. Wright and Douglas [43] gave the name "opsonins" to the active substance which in their opinion is present in normal serum and aids phagocytosis. Opsonins are nonspecific and act to render bacteria sensitive to phagocytosis. Neufeld and Rimpau [44] observed that certain antibacterial serums contained specific antibodies. "Bacteriotropins" is the name applied to these antibodies. Forssman [45] stated that serums from immune animals have no more ability to liquefy staphylococci strongly and efficiently than normal serums. Furthermore, the opsonins are not higher than the normal. However, when this serum is given to rabbits they are protected against a dose of staphylococci which is lethal for the nonimmune animal.

Besredka [46] expressed the opinion that it is difficult to vaccinate against the staphylococcus, and that, furthermore, the staphylococcus does not stimulate production of antibodies. He said, however, that man may derive some benefit from such vaccination. This favorable result of vaccinotherapy in man proves that antistaphylococcic immunity does exist, but the mechanism of it is not understood.

LOCAL IMMUNITY

Besredka [46] apparently has contributed more than any other investigator to the knowledge of this phase of immunity. He showed that dressings soaked in cultures of staphylococci or in staphylococcus filtrates when applied to the skin of guinea-pigs acted as a vaccine. Friedlander and Toomey [47] later found that plain broth was just as effective as specific broth filtrates if used as a skin compress for the protection of guinea-pigs against a subcutaneous injection of Staphylococcus aureus. The immunity is not specific and is localized to the

42. Topley, W. W. C.: Outline of Immunity, Baltimore, William Wood & Company, 1933.

43. Wright, A. E., and Douglas, S. R.: Proc. Roy. Soc., London, s.B **72**:364, 1904.

44. Neufeld, F., and Rimpau, R.: Ztschr. f. Hyg. u. Infektionskr. **51**:283, 1905.

45. Forssman, J.: Acta path. et microbiol. Scandinav., supp. 26, 1936, p. 116.

46. Besredka, A.: Local Immunization, Baltimore, Williams & Wilkins Company, 1936.

47. Friedlander, S. O., and Toomey, J. A.: J. Exper. Med. **47**:663, 1928.

area compressed. The protection lasts at least twenty-four hours after the removal of the compress. Definite histologic changes occur in the skin following this treatment. There are edema, proliferation of clasmatocytes and thickening of the epidermis together with moderate exudation of polymorphonuclear and small mononuclear cells. Bacteria when injected into these immunized areas are phagocytosed early by the clasmatocytes. The fibroblasts rapidly wall off the lesion.

Rabbits show slight and irregular evidence of local immunity of the skin when given staphylococcus vaccines intradermally.[48]

REACTIONS FOLLOWING THE USE OF STAPHYLOCOCCUS ANTIGENS

The local and systemic reactions following the clinical use of staphylococcus vaccines have usually been few and of little consequence. Those following the use of staphylococcus toxoid are more numerous. Murray [22] classified the reactions obtained in a series of 116 cases of staphylococcic infections in which treatment was with toxoid, as follows: local, 10; general, 7; lethargic, 12, and exacerbated, 16.

In discussing these reactions Murray [22] said:

> The injection of staphylococcus toxoid produces a certain amount of local swelling and pain, but most patients speak of it as being only "stiffness". . . . Subcutaneous injection proved definitely more painful than intramuscular, . . .
>
> In 12 cases a curious phenomenon of extreme tiredness or lethargy for about 24 hours has been experienced. This may have been associated with a rise in temperature but in only one case was this taken. The maximum reached was 99.8°F., and it quickly subsided. The patient had already proved very sensitive to diphtheria toxoid-antitoxin. It is also of interest that 16 cases had an apparent exacerbation of their infection following, usually, the first two doses of toxoid. The number of really severe local reactions has been remarkably small. I have given over 1,000 injections and have notes on only 17 causing what the patient considered a really painful arm.

The reactions to the initial injection of toxoid are marked and apparently allergic in origin, according to Conner and McKie, since they have no relation to the amount of antitoxin in the serum when treatment is begun.[27a] Dolman [14] found that many patients had such a slight reaction that it passed off unnoticed. In some cases, however, a bright red circumscribed patch appeared at the site of injection within from eight to twelve hours and reached its maximum extent after from twenty-four to thirty-six hours. After this time the discoloration rapidly faded, so that usually no trace remained after three days. About 10 per cent of Dolman's patients had mild headaches, dizziness or nausea and felt tired on the day following the first few injections of toxoid. More severe reactions are very infrequent, and only one alarm-

48. Klopstock, F.: Ztschr. f. Immunitätsforsch. u. exper. Therap. **86**:213, 1935.

ing reaction has been observed with toxoid. This was reported by Whitby.[29b] He stated that

> one patient . . . exhibited acute anaphylactic shock when a fourth injection was given at an interval of three weeks after the third injection. He was a case of osteomyelitis who had been operated on in the interval. This severe reaction must be extremely rare for I have heard of no other case after inquiring from many other workers with a large experience of toxoid treatment.

Severe reactions have also been observed with the toxoid. Cornbleet and Rattner [30b] noted such reactions in the majority of their cases. In two instances the reactions were systemic, accompanied by a slight rise of temperature, muscle pains and malaise. One reaction observed by Kindel and Costello [30a] was so severe that the patient was confined to the bed.

The clinical studies made with toxin as the antigen show a more severe reaction than was observed in those patients who were treated with toxoid. No reactions have been reported of a severe allergic type.

Experimentally Panton, Valentine and Dix [40a] found no evidence of hypersensitivity to toxin in rabbits. They did note, however, that a rabbit which had received a series of injections of toxin usually reacted to a lesser dose of the cocci than the normal rabbit. This state of hypersensitivity was neither so constantly induced nor of as marked a degree as that observed in rabbits sensitized by the living coccus alone.

Several reactions have been observed following intravenous injections of staphylococcus antitoxin.[49] Joyner and Smith [39a] gave antitoxin intramuscularly to eleven patients, none of whom showed an immediate general serum reaction, although in practically all of them serum sickness developed later.

MECHANISM OF IMMUNITY

The immune responses to the different staphylococcus antigens will be considered separately. Conner and McKie [27a] stated that vaccines made from toxigenic staphylococci contain an exotoxin which probably plays some part in the production of immunity. The failure to obtain any immunologic response to a vaccine may be due to the absence of this exotoxin. Dolman [14] likewise was of the opinion that the good results sometimes obtained with the staphylococcus vaccines are probably chiefly due to the toxin or toxoid present in the particular vaccine.

Antitoxin produced by immunization with either toxin or toxoid neutralizes the leukocidin, hemolysin and lethal factor present in staphylococcus filtrates.[50] Ramon and his co-workers [19b] expressed the

49. Joyner and Smith.[39a] Dolman.[39b] Dolman.[40c]

50. Ramon and others.[19b] Dolman.[39b]

opinion that this antitoxin also protects the cells and tissues against the necrotizing action of the staphylococcus poison and thus makes a less favorable medium for the growth and multiplication of the organisms. The leukocytes in the presence of antitoxin are able to phagocytose the bacteria and débris. Staphylococcus antitoxin is therefore indirectly bactericidal.[19b] Dolman [39b] expressed the opinion that since the staphylococcus antitoxin neutralizes leukocidin it may be said to impart an indirect bactericidal function to this serum. Parish and Clark [40b] expressed the belief that the action of staphylococcus antitoxin is mainly antitoxic and said "it may be that it can combat only the toxic attack of the coccus and not its pyogenic assault." No amount of antitoxin subsequently added to the circulation will influence the lesions already produced by the staphylococcus and its toxin.

Ramon and his associates [19b] considered the formation of protective antibodies the result of a stimulus produced by either toxin or toxoid. These antibodies neutralize in vivo the toxin produced by the bacteria.

Studies have been made on the tissues in the normal and the immune animal and also of the process by which bacteria are removed from the blood stream.[51] Staphylococci localize in the liver and spleen in both the normal and the immune animal in far greater numbers than in any of the other organs.[51b] Staphylococci are also removed from the blood stream more rapidly in actively immunized rabbits than in normal ones. There is proliferation of the lymphoid and the histiocytic elements especially in the spleen, liver, bone marrow and lungs of the immune animals. These structural and functional conditions within the organs may explain many of the phenomena of bacterial localization. Thus, in the liver and spleen, the relative concentration of macrophages indicates a common structural relationship, and the sinusoidal type of blood flow suggests a functional similarity. The combination of these two factors should furnish conditions favorable for the approximation of phagocytes and particles in the blood stream.[51a]

Manwaring and Fritschen [51b] showed that the "microbic-tissue affinity" for the staphylococcus was greater in the perfused liver and spleen of the normal dog than in any of the other tissues. In using bacteria other than staphylococci it was observed that this affinity was increased through immunization. Furthermore, the microbic affinity of the immune liver was also increased by the addition of immune serum to the perfusing fluid. "Microbic-hepatic affinity" of a normal liver could be produced by the addition of immune serum to the perfusing fluid so

51. (*a*) Sullivan, F. L.; Necherman, E. F., and Cannon, P. R.: J. Immunol. **26**: 49, 1934. (*b*) Manwaring, W. H., and Fritschen, W.: ibid. **8**:83, 1923.

that it was practically identical to that of an immune liver. The serum component apparently increases this bacterial affinity by acting on the micro-organism.[51b]

Forssman [45] in discussing staphylococcic immunity said that the immunity develops so slowly that it does not correspond to any of the known antibody curves. Because of this fact Forssman thought that one must postulate an unknown antibody.

SUMMARY

In this review of staphylococcic immunity it has been found that vaccines have very little therapeutic effect on staphylococcic infections either experimentally or clinically. It is suggested, however, that they may stimulate the formation of antibodies in man.

Toxoid appears to be a very efficient antigen in the treatment of certain staphylococcic infections of low virulence in the skin and may be of some value in certain cases of chronic infections, such as osteomyelitis. Toxoid produces an increase in the circulating antihemolysins, and this is frequently accompanied by clinical improvement.

The experimental and clinical results suggest that staphylococcus antitoxin has a definite therapeutic value in treatment in certain cases of acute staphylococcic infections which are accompanied by toxemia. This antitoxin may act in the following ways: The toxin produced by the staphylococcus in vivo is neutralized by this antitoxin; a component of the immune serum may act on the staphylococci and increase the ease with which they are phagocytosed by certain organs, especially the liver and spleen. The antitoxin in the circulation may also inhibit the action of leukocidin and thus allow the polymorphonuclear leukocytes to phagocytose the bacteria in either the blood or the tissue.

From the experimental observations on the mechanism of immunity to staphylococcic infections it appears that a vaccine, toxin or toxoid may also cause proliferation of the lymphoid and histiocytic elements, especially in the liver and spleen. This cellular change enhances the ability of these tissues to phagocytose the staphylococci.

Notes and News

Society News.—The twenty-second annual session of the American College of Physicians will be held in New York City, April 4-8, 1938, with headquarters at the Waldorf Astoria Hotel.

The American Society of Clinical Pathologists has elected the following officers: president-elect, T. B. Magath; vice president, Stanley P. Reimann; secretary, A. S. Giordano.

The newly elected officers of the Section on Pathology and Physiology of the American Medical Association are: Roy R. Kracke, chairman; M. B. Visscher, vice chairman; J. J. Moore, secretary.

The Society of American Bacteriologists will hold its next annual meeting in the Mayflower Hotel, Washington, D. C., Dec. 28-30, 1937. The meeting in 1938 will be held in San Francisco, Calif., Aug. 30-Sept. 1, 1938.

The newly elected officers of the American Association for Cancer Research are: president, James Ewing; vice president, G. H. A. Clowes; secretary-treasurer, A. A. Thibaudeau.

Arrangements are under way for holding the fourth International Leprosy Conference in Cairo, beginning March 21, 1938. The Egyptian government is inviting the countries concerned to send official delegates. Physicians and others who are interested in leprosy will be welcome. For further information, address the secretary of the International Leprosy Conference, 131 Baker Street, London, W. I.

New Unit at Bellevue Hospital.—To meet the growing needs of the municipal hospitals of New York City for laboratory facilities for the diagnosis and study of tropical diseases, a new unit for that purpose has been established at Bellevue Hospital.

University News, Promotions, Resignations, Appointments, Deaths, etc.—In the University of Alabama, Oscar O. Christianson, instructor in pathology in Rush Medical College, University of Chicago, has been appointed assistant professor of bacteriology and pathology in the place of Cornelius S. Hagerty, who will take up private practice.

Harry S. Mustard, associate professor of public health administration in the School of Hygiene and Public Health of the Johns Hopkins University, has been appointed professor of preventive medicine in New York University, succeeding William H. Park, retired.

Emmerich von Haam, assistant professor of pathology in Louisiana State University, has been appointed chairman of the department of pathology of the Ohio State University.

Herbert K. Fidler has been promoted to the post of assistant professor of bacteriology and pathology in the school of medicine of the University of Alabama.

G. Albin Matson, associate professor of bacteriology at the Montana State University, has been appointed to an assistant professorship in the department of bacteriology and pathology of the school of medicine of the University of Utah.

R. B. H. Gradwohl has resigned as director of the laboratory of the St. Louis County Hospital. His successor is H. N. Allen.

L. H. Snyder has been appointed instructor in pathology in George Washington University, Washington, D. C.

Florence B. Seibert, professor of biochemistry in the Henry Phipps Institute of the University of Pennsylvania, has been awarded a Guggenheim fellowship to make a study with the ultracentrifuge of the molecular sizes and cataphoretic mobilities of the active principle of tuberculin in its antigenic and in its non-antigenic form, to be carried out in the laboratory of Th. Svedberg at the University of Uppsala.

Abstracts from Current Literature

To Save Space the Original Titles of Abstracted Articles Sometimes Are Shortened

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Experimental Pathology and Pathologic Physiology

The Mechanism of Increased Capillary Permeability in Inflammation. V. Menkin, J. Exper. Med. **64**:485, 1936.

Various types of inflammatory exudates have been obtained in dogs and rabbits by introducing a chemical irritant into normal tissues, by burns or by injecting bacteria. An exudate of human origin also has been studied. These exudates have all been found to contain a factor which induces prompt increase in the permeability of the capillaries of normal skin. This factor is demonstrable by an almost immediate accumulation of trypan blue from the circulation into areas of skin where injections of cell-free exudate have been made. The active factor may be carried down with the precipitate resulting from the interaction of the exudate with either ammonium sulfate, added to the point of saturation, or sodium sulfate in the concentration of 20 per cent. The active factor passes through a dialyzing membrane. It can be recovered from the dialysate as a protein-free crystalline material. The active factor manifests no property in common with histamine or, presumably, with the hypothetic H substance, assumed to be closely related to histamine. This is indicated by: (*a*) the difference between the tissue staining pattern of the exudate or of its active fraction and that of histamine; (*b*) the opposite effects by histamine and the active factor on the tonicity of an isolated strip of guinea-pig intestine. The observations presented in this report do not substantiate Lewis' hypothesis that histamine or the closely related H substance is the primary cause of the increase in capillary permeability in inflammation.

From the Author's Summary.

Cyclic Changes in the Human Vaginal Mucosa. H. F. Traut, P. W. Bloch and A. Kuder, Surg., Gynec. & Obst. **63**:7, 1936.

The matter of whether or not the human vaginal mucosa exhibits cyclic changes like that of the uterus and oviducts has long been controversial. The authors found that previous observers had based their opinions on too few biopsies or that the subjects were not normal. Accordingly they obtained four or more specimens of vaginal mucosa, always from the same part of the wall, from twenty-nine women with normal menstrual cycles. The histologic studies indicate the existence of a definite rhythm in the vaginal mucosa which can be correlated with menstruation and hence with the ovarian cycle. There occurs a cellular response, characterized by proliferation on the part of the stratum germinativum with increase in the number of young epithelial cells in the basophilic zone of the epithelium, occasional mitoses and very definite leukocytosis and hyperemia. The proliferative phase appears in the premenstruum, continues for from six to seven days and is either completed before menstruation or extends into this phase and occasionally into the postmenstruum, It was not found between the seventh and twenty-first days of the cycle. A quiescent phase exists between the periods of proliferation. Another observation which seems to indicate that the rhythm of the vagina is related to the ovarian cycle is the absence of alternate proliferative and inactive phases of pregnancy. Unlike certain previous writers, the present authors were unable to correlate changes in the superficial and intra-epithelial layers of the vaginal mucosa with the menstrual cycle.

Warren C. Hunter.

INFLUENCE OF THE ENDOCRINE GLANDS ON THE SKELETAL SYSTEM. G. CORYN, Arch. internat. de méd. expér. **11**:135, 1936.

There exists no positive proof of the influence of the parathyroids on the proliferation and hypertrophy of cartilage cells or of their importance in ankylosing arthritis. The effect of the parathyroids on the calcification of the skeleton has been well shown, but it is probable that they do not exercise any influence over other phases of endochondral osteogenesis. The term "fibrocystic osteitis" is inadequate to express the similarity of lesions of hyperparathyroidism. It does not describe exactly the ultimate stage of the disease. The fibrosis of the marrow, the cysts and the brown tumors are not characteristic. The fibrosis of the marrow represents a common reaction of osseous tissue against various factors. Study of the elimination of calcium in the urine gives more constant results than study of the metabolism of calcium. The parathyroids are not important in the etiology of Paget's disease. A study of seven cases of osteopsathyrosis, of which two occurred in children and five in adults, emphasized the similarity of this disease to hyperparathyroidism of the adult. This conclusion is based on the variations in blood calcium and blood phosphorus in both conditions. It is probable that the parathyroids partake in the genesis of osteopsathyrosis. Studies of the blood phosphatose in a large number of patients suffering from various disorders led to the conclusion that in bone disease the factor that makes the blood phosphatase vary is the proliferation of connective tissue and not the decalcification of the skeleton. A case of hypoparathyroidism with hypercalcification of the skeleton is presented.

FREDERICK STENN.

EXPERIMENTAL LYMPHOGENOUS HYPERERGIC APPENDICITIS. E. FISCHER and H. KAISERLING, Virchows Arch. f. path. Anat. **297**:146, 1936.

This detailed study of the hyperergic reaction of the vermiform appendix of the sensitized rabbit places acute appendicitis in the category of the allergic diseases. The animals were sensitized by repeated subcutaneous injections of swine serum. In order that the lymphatics might be more carefully studied, they were made more prominent by injecting a small quantity of air into the submucosa at the tip of the appendix. The air distends the perifollicular lymphatic plexus and passes from here into the lymphatics of the various coats of the appendix and into the efferent lymph vessels. With the lymphatics the authors do not include the perivascular and tissue spaces, which do not have an endothelial lining. The injection of air immediately preceded the injection into the submucosa at the tip of the activating dose of the antigen. The appendixes were removed for histologic study at intervals of from one hour to six days after injection of the antigen. Within an hour the lymphatics were filled with coagulated plasma; attempts to inject air into the vessels at this stage were unsuccessful. The serosal blood vessels became engorged; after a further period of from four to six hours the serosal hyperemia disappeared except in a circular segmental area in the distal half of the appendix but not at the point of injection of the air and antigen. The lymphatic endothelium swelled, the walls underwent fibrinoid swelling and degeneration, and the vessels were surrounded by a cellular infiltration of lymphocytes, leukocytes and plasma cells. In the segmental area of persisting hyperemia, small areas of necrosis developed in the mucosa and the structure of the lymphoid follicles was disrupted. Bacterial invasion now occurred, and the inflammatory process became more intense and spread throughout the appendix by way of the lymphatics and tissue spaces; in some animals acute purulent peritonitis developed. Injection of air and serum into the submucosa of the appendix of nonsensitized control animals led to only very slight reaction of the lymphatics, which did not progress to hyperergic reaction and infection as in the sensitized animals. On the basis of their experimental findings the authors set up the hypothesis that human acute appendicitis occurs in persons who have become sensitized to some specific protein. The appendix and especially its lymphatic structures are in a state of potential reactivity to a subsequent entrance of the antigen. The reactivity of the allergic

person may manifest itself as a hyperergic reaction of the appendix, which leads to bacterial invasion and infection. Aschoff has maintained that the bacterial incitants of acute appendicitis are usually the normal inhabitants of the intestinal tract. But it has been difficult to explain the sudden acquisition of virulence by such organisms. Ricker has held a neurovascular reaction that leads to stasis to be the underlying factor. Fischer and Kaiserling found that extirpation of the celiac ganglion led to a more intense and more rapidly progressing hyperergic response on the part of the appendix. They believe that the contentions of both Aschoff and Ricker find an explanation in the hyperergic character of the early reaction of acute appendicitis.

O. T. Schultz.

Anatomic Changes in the Hearts of Dogs and Frogs Poisoned by Glucosides of the Digitalis Group. C. H. Hu, V. T. Lieu and R. C. Li, Chinese M. J., supp. 1, 1936, p. 31.

In seventeen of nineteen hearts of dogs dying of cumulative poisoning by glucosides of the digitalis group, focal necroses are present in the endocardial side of the myocardium, chiefly of the left ventricular wall and in the papillary muscles. These lesions, which are practically entirely absent in the hearts of twenty normal control dogs, may be secondary to the ischemic condition of the myocardium brought about, probably, by the vasoconstrictor action of the drugs on the coronary arteries. The important anatomic change in the hearts of frogs given injections of large doses of digitoxin, lanadigin and ouabain consists of marked hyalinization of the myocardium. This change is apparently due to the marked contraction of the muscle fibers under the influence of the drugs. It is observable chiefly in animals dying within seven hours after receiving the injections. Animals dying many hours after receiving the injections do not, as a rule, show this change. Contrary to the findings in the hearts of the dogs, no focal necrosis of the myocarium is found in the hearts of frogs. This might be explained by the absence in the frog's myocardium of the coronary arteries on which the drugs may act to cause the constriction that results in ischemia and necrosis.

From the Authors' Summary.

Pathologic Anatomy

Massive Calcification of the Myocardium. J. A. Redfearn, Am. Heart J. **12**:365, 1936.

The case of calcification of the myocardium reported here is of interest because the process was so extensive as to involve the entire anterior half of the left ventricle, because the history extended over a period of nine years and because vascular channels were demonstrated in the pericardial adhesions, a condition similar to that found in the cases reported by Beck, Tichy, Hirschboeck and others.

From the Author's Summary.

The Nature and Significance of the Structural Changes in the Lungs in Mitral Stenosis. F. Parker and S. Weiss, Am. J. Path. **12**:573, 1936.

The study of the lungs in five cases of advanced rigid mitral stenosis associated with intense failure of the pulmonary circulation revealed that the lesions in the pulmonary vessels consisted of intimal thickening in the arteries and hyperplastic arteriolar sclerosis and arteriolar necrosis. The changes in the alveolar walls consisted of marked dilatation of the capillaries, increase in the thickness of the capillary basement membrane, increase in the interstitial collagenic tissue, presence of interstitial capillary edema and a tendency of the flat epithelial cells to become cuboid. Despite advanced thickening of the alveolar wall and of the capillary basement membrane, the alveolar basement membrane remained normal. Progres-

sive pulmonary engorgement showed, at first, an increase in the number, and later dilatation, of the capillaries with pericapillary and intra-alveolar edema of varying degrees. These advanced vascular lesions developed within a period of about two months.

FREDERICK STENN.

THE EVOLUTION AND INVOLUTION OF THE PROSTATE GLAND. R. A. MOORE, Am. J. Path. **12**:599, 1936.

At puberty there is rapid maturation of the prostate, probably due to an internal secretion of the testis, which is activated by the pituitary gland. The mature postpubertal prostate is maintained as a uniform structure for about twenty-five years, except for occasional instances of pathologic involution dependent on systemic disease. During the fifth decade of life involution is initiated which continues into the eighth decade. All the evidence indicates that this involution is the result of a decrease in and cessation of the same internal secretion which appeared at puberty. There are occasional instances in which senile involution is delayed for from ten to twenty years beyond the average time. There is no adequate morphologic explanation for this. During the presenile period a nonuniformity of structure is characteristic and is probably the result of irregular stimulation combined with unequal reactivity of the tissues.

FROM THE AUTHOR'S SUMMARY.

CERTAIN CYTOPLASMIC INCLUSIONS OF LIVER CELLS. A. M. PAPPENHEIMER and J. J. HAWTHORNE, Am. J. Path. **12**:625, 1936.

Certain spherical, rodlike and filamentous cytoplasmic inclusions occur frequently in the liver cells of man, monkeys, ferrets and guinea-pigs. They have not been seen in the livers of other laboratory animals. No decision has been reached as to their nature and significance.

FROM THE AUTHORS' SUMMARY.

ORIGIN OF SENILE PLAQUES IN THE CEREBRAL CORTEX. S. HIROISI and C. C. LEE, Arch. Neurol. & Psychiat. **35**:827, 1936.

The origin and nature of senile plaques are matters of great controversy, and for this reason ideas relative to their histogenesis are numerous. Hiroisi and Lee think that the plaques result from mucinoid degeneration of oligodendroglia, which when degenerated give the same selective stain with Mayer's mucicarmine. Degenerated oligodendroglia cells give rise to minute senile plaques, which represent an early stage of the process of which the senile plaques are the final outcome.

G. B. HASSIN.

CALVARIAL HYPEROSTOSIS. SHERWOOD MOORE, Arch. Neurol. & Psychiat. **35**:975, 1936.

The calvarium may exhibit excessive growth of bone, causing thickening or hyperostosis. Moore differentiates four types of hyperostosis of the calvarium, and of these he considers that of the frontal bone most characteristic. Here the overgrowth of cancellous bone lies on the inner table without evidence of an inflammatory process. Occasional extensions to other bones, for instance, those of the base of the skull, may take place. In the other three types of hyperostosis the process is confined to the diploe. Whatever the type, there is bilateral symmetry of the osseous changes, and no changes are seen in the outer table of the skull, which is not increased in size. The symptoms may be headache, obesity, epileptiform seizures, visual disturbances and mental slowness.

G. B. HASSIN.

SIMILARITIES BETWEEN SOME FORMS OF "ENCEPHALOMYELITIS" AND MULTIPLE SCLEROSIS. TRACY J. PUTNAM, Arch. Neurol. & Psychiat. **35**:1289, 1936.

Putnam described the changes in the myelin, axis-cylinders and blood vessels in the plaques of multiple sclerosis, disseminated acute encephalomyelitis and the patches in the brain which he produced experimentally by venous obstruction. He concluded that a relationship exists between the acute forms of multiple sclerosis and encephalomyelitis and that histologic features of the latter may be seen in the more acute plaques of multiple sclerosis. The histologic differences between the two conditions are due, in his opinion, to time, multiple sclerosis being a chronic, encephalomyelitis an acute or subacute, condition. The foci seen in postvaccinal and postmeasles encephalopathies are, in Putnam's opinion, acute forms of the typical plaques seen in multiple sclerosis. G. B. HASSIN.

THE CHANGES IN THE BRAIN IN PUERPERAL ECLAMPSIA. I. B. DIAMOND, Arch. Neurol. & Psychiat. **35**:1320, 1936.

The condition of the liver and kidneys in puerperal eclampsia denotes a severe toxic state; this may be affirmed also from the histologic changes in the brain, which Diamond studied in five cases. Though the changes varied somewhat in each case, some could be classified as fundamental, for they occurred in each case. Such changes were rarefaction or edema of the brain tissues and various degenerative changes in the ganglion cells (from swelling to liquefaction), scattered throughout the brain. Other changes were termed by Diamond focal or accidental. They were glia nodules, infiltrations of blood vessels, perivascular hemorrhages and marked regressive changes in the glia. Of especial interest was the reaction and phagocytic activity of the microglia. Though this was noted in every case, it was occasionally especially marked. The microglia showed numerous sizes and shapes; some resembled rod cells or fat granule bodies, and contained red cells in vacuoles. Another feature was the meningeal reaction—the presence in the subarachnoid space of a great variety of cells, namely, histiocytes, fibroblasts, mesothelial cells and occasionally lymphocytes—a condition described as aseptic meningitis. G. B. HASSIN.

THE DISPOSAL OF BARIUM SULFATE IN THE ABDOMINAL CAVITY. J. C. THOMAS, J. Path. & Bact. **43**:285, 1936.

The introduction of barium sulfate particles ("barium meal") into the peritoneal cavity produces a reaction characterized by a transient nonspecific migration of polymorphonuclear leukocytes, a marked response of lymphocytes and macrophages and later encapsulation of the foreign substance with granulation tissue. Most of the barium sulfate particles are phagocytosed by macrophages. Foreign body giant cells are formed only in the very late stages. The response to barium sulfate appears to differ from the classic reaction to foreign bodies in the intense and persistent lymphocytic and polyblastic reaction and the delayed formation of giant cells. FROM THE AUTHOR'S SUMMARY.

TUBERCULOUS VERRUCOSE ENDOCARDITIS. T. B. DAVIE, J. Path. & Bact. **43**:313, 1936.

A brief review of the literature on tuberculous endocarditis shows that the condition is relatively rare. A verrucous mitral and aortic endocarditis believed to have been of tuberculous origin is described, with macroscopic and microscopic illustrations. The criteria previously regarded as essential for the diagnosis of this lesion are discussed and apparent fallacies in them pointed out. A new hypothesis to account for the mode of production and for the rarity of the condition is put forward. This theory assumes the existence of a tuberculous allergic endocarditis similar in type to rheumatic endocarditis and postulates the rare

coincidence of tuberculous bacillemia during the phase of the allergic endocarditis to explain the accepted form of tuberculous bacterial endocarditis. The latter condition is thus brought into line etiologically with subacute bacterial endocarditis.

FROM THE AUTHOR'S SUMMARY.

THE MORPHOLOGIC SIGNIFICANCE AND DEVELOPMENT OF MALFORMATIONS OF THE HUMAN HEART. H. BREDT, Virchows Arch. f. path. Anat. **296**:114, 1935.

This is the first of a series of contributions on cardiac maldevelopments based on the material of Rössle's institute since 1931. In it Bredt attempts to evaluate the embryogenesis of certain maldevelopments in the light of their morphology and of current and often controversial theories. In the first section he describes hearts in which two or more anomalies were combined and seeks to determine whether such multiple malformations are a necessary result of some primary failure of development in the sense that a septal defect, for instance, may be secondary or compensatory to stenosis of an ostium, or whether the multiple maldevelopments may be brought about by a factor acting independently on different parts of the developing organ. In the second section he deals with atresias and stenoses of the cardiac cavities and ostia. In the third section he offers a classification of the forms of so-called persistent truncus arteriosus communis, and expresses the belief that when the trunk has a valve of three rather than four segments the anomaly is the result of suppression of the pulmonary artery or aorta and not of simple persistence of an originally single truncus arteriosus. In the fourth section are described some unusual auricular defects, such as an unusual defect of the septum, congenital bands of the interior of the auricles, and atresia of the mouth of the coronary sinus. The final section relates to failures of development of the coronary arterial system.

O. T. SCHULTZ.

RETRACTILITY AND DISTENSIBILITY OF THE GREAT SAPHENOUS VEIN. R. NEUMANN, Virchows Arch. f. path. Anat. **296**:158, 1935.

In seventy-seven unselected necropsies of persons varying in age from 6 to 93 years, the great saphenous vein together with surrounding connective tissue, fat, muscle and overlying skin was removed from the mouth of the vein to the level of the knee joint. The spontaneous retractility of the vessel was measured, and then, by means of a specially devised apparatus, the length, diameter and volume of the vein after distention with physiologic solution of sodium chloride under pressures of 12.5, 25, 50, 100, 200, 300 and 400 mm. of mercury were determined. The pressure was allowed to act for ten seconds, the changes being recorded on a kymograph. Spontaneous retractility decreased with advancing age; the shortening varied from 39 per cent at 6 years to 6 per cent at 93. Distention of the vessel under low pressure led to increase in length with slight change in diameter. With increasing pressures the changes noted occurred in five successive phases: increase in length with slight increase in diameter; further increase only in length; then increase only in diameter; marked distention with shortening, and finally distention with increase in length. In persons with edema, varices, cachexia and obesity these phases followed each other quickly; the change was one chiefly of increase in length, and the vein was relatively nonresistant to rupture under high pressures. In persons with thrombosis or atherosclerosis and in those in the lower and higher age periods, the phases followed each other more slowly, and the vessel was highly distensible and more resistant to rupture. The differences noted are ascribed chiefly to the condition of the perivascular tissues. If the latter are edematous or contain much fat the vein is less resistant. The time of removal of the vein, at intervals varying from two hours to sixteen days, had no influence on the properties investigated.

O. T. SCHULTZ.

HYDROPIC DEGENERATION OF INTESTINAL SMOOTH MUSCLE. K. HELMKE, Virchows Arch. f. path. Anat. **296**:192, 1935.

As hydropic degeneration Helmke describes a condition in which the smooth muscle cells are swollen as the result of the presence of an increased amount of intracellular fluid. The muscle fibrils are displaced and compressed. An increase in intercellular fluid may lead to compression atrophy of smooth muscle. Both forms of degeneration have been observed chiefly in the inner muscle coat in conditions in which the intestinal contents are abnormal. The outcome is held to be sclerosis of the inner muscle coat. O. T. SCHULTZ.

INCIDENCE OF CHOLANGEITIS IN RELATION TO CHOLECYSTITIS. S. LAMANNA, Virchows Arch. f. path. Anat. **296**:240, 1935.

In 300 selected necropsies LaManna encountered 159 cases of cholecystitis. The greatest frequency was in the two decades from 50 to 69 years. Gallstones were present in 122 of the 159 cases of cholecystitis. To determine the relationship between cholecystitis and intrahepatic cholangeitis, a microscopic examination was made of various portions of the liver. Cholangeitis was present in 75 of the 159 cases of cholecystitis; in 30 the process was of mild grade. Dilatation of the intrahepatic ducts, indicative of a more severe degree of involvement, was most marked when the outflow of bile was obstructed by a tumor. The necropsy material included 14 cases in which there was intrahepatic cholangeitis without involvement of the extrahepatic biliary passages. O. T. SCHULTZ.

INCREASING INCIDENCE OF EMBOLISM IN RELATION TO AGE. K. H. ZINCK, Virchows Arch. f. path. Anat. **296**:289, 1935.

Statistical analysis of the necropsy material of Rössle's institute reveals a definite increase in the incidence of thrombosis and embolism since 1924 but does not uphold the view that this increase is correlated with an increase in the number of persons reaching the higher years of life or with an increase in carcinoma or other diseases that may be looked on as results of the aging of the tissues. O. T. SCHULTZ.

NEW FORMATION OF LYMPHOID TISSUE IN MYCOSIS FUNGOIDES. K. H. ZINCK, Virchows Arch. f. path. Anat. **296**:319, 1935.

Necropsy of a 63 year old man with the external lesions of mycosis fungoides revealed granulomatous nodules of characteristic histologic appearance in the lungs, heart, stomach, duodenum, right adrenal gland and inguinal lymph nodes. The vegetative nervous system, the peripheral nerves and the organs of internal secretion with the exception of the right adrenal gland were not involved. In the histologic examination especial attention was paid to the reticulum of the granulomas. Most previous investigators have held that the reticulum of the granuloma is formed by splitting of the preexisting connective tissue by the infiltrating cells. Zinck's studies lead him to conclude that the reticulum is newly formed and is an essential part of the granulomatous tissue. He believes that the latter is derived from perivascular indifferent mesenchyme, proliferation of which results in differentiation along various lines. Such a process would account for the formation of reticulum by reticulo-endothelial cells and for the presence of the lymphocytes, plasma cells and other cellular elements of the granuloma. O. T. SCHULTZ.

THE HISTOLOGY OF BONE IN OSTEOSCLEROSIS FRAGILITIS. W. LAUBMANN, Virchows Arch. f. path. Anat. **296**:343, 1935.

A man aged 25 years had had rickets in childhood, followed later by a protracted siege of hip joint suppuration. In more recent years he had had spon-

taneous fractures. Roentgenographic examination revealed throughout the skeleton the findings characteristic of Albers-Schönberg disease, or marble bones. Anemia became severe before death. Laubmann presents a description of the histologic changes of various bones. In the gross it was difficult to distinguish between cortical and cancellous bone because of the eburnation of the latter. The primary bone formed by calcification of the original cartilaginous matrix is not resorbed in a normal manner but persists. On the surface of the calcified cartilaginous trabeculae osteoid tissue is laid down. The latter may penetrate the persisting cartilaginous bone. In time it becomes partly or completely ossified. It replaces the haversian canals, and the marrow undergoes fibrosis. In the case described, small islands of cellular regenerating marrow persisted, but the greater part of the marrow had been replaced by fibrous tissue. Larger fibrous areas may undergo chondroid or osteoid metaplasia, with later calcification or ossification. The marbled appearance of the bone is due to the formation in the cancellous bone of rounded masses termed "osteones" by Laubmann, which are composed of concentric laminae of dense bone. He considers the process to be one primarily of greatly delayed resorption of calcified cartilage associated with continuing formation of such cartilage, leading to marked disturbance of enchondral ossification. Ossification of the membranous bones of the skull was also abnormal and resulted in replacement of the diploe.

O. T. SCHULTZ.

GIBBUS FORMATION IN VERTEBRAL HODGKIN'S DISEASE. H. BEITZKE, Virchows Arch. f. path. Anat. **296**:358, 1935.

Involvement of the vertebral column in lymphogranulomatosis is not unusual, but formation of a gibbus, according to Beitzke, is rare. Usually several vertebral bodies are destroyed, but the intervertebral disks are preserved; hence the spine is not angulated. The case reported was that of a woman aged 32 years. Necropsy revealed lymphogranulomatosis of the bronchial and mediastinal lymph nodes and thymus with direct extension to the myocardium and metastasis to the right lobe of the thyroid gland, diaphragm, stomach and surface of both kidneys. The bodies of the seventh cervical and first thoracic vertebrae and the adjacent disks had been destroyed. The two vertebral bodies had been transformed into wedge-shaped remnants in which the thinner portions were directed anteriorly. The result was an angulation of the spine of 145 degrees. The cervical portion of the spine was displaced forward. The spinal cord was not involved.

O. T. SCHULTZ.

HISTOLOGY OF THE VAGUS AND SYMPATHETIC GANGLIONS IN TYPHUS. E. HERZOG, Virchows Arch. f. path. Anat. **296**:403, 1935.

The superior cervical ganglion was examined histologically in eighty necropsies, and the vagus ganglion in fifty. The material was derived from the 1932-1935 epidemic of typhus in Chile, in which there was a mortality of 15 per cent. In 87 per cent of the cases the sympathetic ganglion and in 84 per cent the vagus ganglion revealed small perivascular infiltrations by leukocytes, lymphocytes, histiocytes and plasma cells. Hyperemia of the vessels, with small hemorrhages and occasional thrombosis of small vessels, was also observed. In ten cases the infiltrative process was diffuse. Herzog believes that the changes noted explain the symptoms of involvement of the vegetative nervous system, and that the fleeting character of these symptoms in persons who have recovered is explained by the slight degenerative changes observed in the ganglion cells.

O. T. SCHULTZ.

REGENERATION OF THE CAPILLARY BASEMENT MEMBRANE. K. HUECK, Virchows Arch. f. path. Anat. **296**:416, 1935.

The findings reported are based on a study of young granulation tissue. The capillary is described as consisting of an internal cellular membrane, the endo-

thelium; external to this a nonnucleated basement membrane, and external to the latter another cellular membrane, composed of mesenchymal cells (pericytes, clasmatocytes, fibrocytes). In regenerating capillaries the basement membrane appears first as a jelly-like protoplasmic substance, which is formed by the cells, but whether from the endothelium alone or from the outer layer of cells alone or from both could not be determined. The formation of fine fibrils in the intercellular substance gives it a firmer consistency. Its transformation into a thin-walled cylinder depends on its spatial relation to the endothelium, on one side, and the outer cellular layer, on the other.

O. T. SCHULTZ.

THE HISTOLOGY OF HYPERTROPHIED TONSILS. A. VON SZÜTS, Virchows Arch. f. path. Anat. **296**:557, 1936.

The tonsils of 200 persons of ages from a few months to 50 years were examined histologically. Hypertrophy was observed most often in the age group of from 5 to 15 years. The process was always associated with evidences of inflammation in the tonsils. The basal cell layer of the epithelium of the crypt underwent hyperplasia. Reticulation and desquamation of epithelium followed. The reticulum of the tonsil was broken and in part had disappeared. This process is ascribed to the action of ferments liberated by the reticulocytes. The collagenous connective tissue was increased.

O. T. SCHULTZ.

Pathologic Chemistry and Physics

COMPARATIVE CHEMICAL AND HISTOLOGIC EXAMINATION OF THE AORTA FOR CALCIUM CONTENT. S. R. HAWTHORN and others, Am. J. Path. **12**:283, 1936.

Fifty-two aortas from patients between the ages of 2 months and 78 years were analyzed chemically for metallic calcium, and the results compared with those of microchemical tests for calcium salts on the same aortas, with the following conclusions: None of the recommended microchemical tests for visible calcium gives more than a vague idea of the amount that can be recovered from the same aorta chemically. Von Kossa's silver method does not yield a specific stain for calcium, but for microscopic tests it is the most satisfactory indicator of the comparative amounts of calcium deposited in sclerotic lesions. Deposits of calcium were brought out microchemically in only one of twenty aortas from persons under 40 years of age, in 75 per cent of aortas from persons over 40 years of age, and in 100 per cent of those from persons over 60 years. As age advances there is a consistent increase in the calcium content of the aorta in excess of that of the bodily tissues generally. Mild intimal lesions may occur without an increase in calcium as determined by chemical analysis. When the calcium values for the various segments of all aortas from persons over 40 years of age are averaged, it is found that the heaviest calcium deposits are in the abdominal portions. In advanced aortic arteriosclerosis there is constantly an overlapping of "type lesions," such as atheromatous cysts, diffuse medial calcification and plaque deposits. In this, of all groups of lesions, the greatest inconsistencies between chemical and microscopic results occur. For very advanced sclerotic lesions, characterized by heavy platelike calcium deposits, the chemical analyses yield the highest calcium values, and the amounts are most nearly consistent when any two given sclerotic aortas are compared. If the amounts of calcium obtained by chemical analysis are to be correlated with those which can be shown microscopically in sclerotic aortas, additional knowledge is required.

FROM THE AUTHORS' SUMMARY.

THE CALCIUM CONTENT OF THE BLOOD SERUM DURING AN EPILEPTIC CONVULSION. M. SCOTT and A. W. PIGOT, Arch. Neurol. & Psychiat. **36**:590, 1936.

In fifty cases of chronic epilepsy, the authors determined the calcium in blood serum obtained during the epileptic seizure and contrasted the results with those

obtained in serum taken from the same patients during the free intervals. The method of Clark and Collip was used. In 93 per cent of the determinations, the calcium level was from 9 to 14 mg. per hundred cubic centimeters; that is, it was either normal or indicative of hypercalcemia. No appreciable difference was found in the calcium content during the convulsion and between convulsions.

GEORGE B. HASSIN.

THE RELATIVE GLYCOGEN CONTENT OF THE MYOCARDIUM AND THE CARDIAC CONDUCTION SYSTEM. A. NOLL and M. BECKER, Virchows Arch. f. path. Anat. **296**:443, 1935.

Contrary to the findings of Buadze and Wertheimer and in conformity with those of Yamazaki and of Yater, the authors found that the conduction system of the heart of the horse and that of the calf contain more glycogen than the myocardium.

O. T. SCHULTZ.

BARIUM IN THE EYE AND IN MELANOTIC TUMORS. W. GERLACH and R. MÜLLER, Virchows Arch. f. path. Anat. **296**:588, 1936.

Previous spectrographic analyses had led the authors to state that barium does not occur normally in human tissues, although strontium and aluminum are regularly present. In further work they found that barium is present in the eye, being localized chiefly in the choroid, and in melanotic tumors.

O. T. SCHULTZ.

Microbiology and Parasitology

SPOTTED FEVER—FIÈVRE BOUTONNEUSE. G. M. HASS and H. PINKERTON, J. Exper. Med. **64**:601, 1936.

Several methods for the experimental study of the rickettsial diseases have been applied to fièvre boutonneuse. An analysis of the results has indicated that fièvre boutonneuse is a variety of spotted fever and that the etiologic agent is a rickettsia which belongs to the genus Dermacentroxenus and to the species D. rickettsii. The distinctive morphologic aspects of the organism and its characteristic intranuclear clustering in ticks and in tissue cultures are the important criteria on which this conclusion is based. Immunologic, histologic and cytologic observations of a confirmatory nature are also reported.

FROM THE AUTHORS' SUMMARY.

CORYZA OF THE DOMESTIC FOWL: COCCOBACILLIFORM BODIES. J. B. NELSON, J. Exper. Med. **64**:749 and 759, 1936.

The coccobacilliform bodies of fowl coryza were successfully cultivated in the fetal membranes of fertile eggs. Microscopic examination indicated growth in approximately 50 per cent of ninety-four eggs inoculated on the third to fourth day of incubation. Growth was generally inhibited, however, in eggs inoculated on the tenth day. One strain of the specific bodies was maintained through eleven successive passages in four day eggs. A more consistent growth of the coccobacilliform bodies was obtained in tissue cultures. One strain, originally isolated in November 1935, has been carried through a hundred subcultures at intervals of from one to three days. The specific bodies fail to maintain their morphologic identity for any length of time in this medium. It is noted that growth of the coccobacilliform bodies in fertile eggs and in tissue cultures is not dependent on the presence of living cells.

Fetal membrane and tissue culture suspensions of the coccobacilliform bodies are infective for normal fowl. Intranasal injection is commonly followed by a coryza which is serially transmissible and communicable by direct contact. The specific bodies are generally demonstrable in the nasal exudate of birds infected

by injection or by contact. Compared with the original strain of the coryza of slow onset, these suspensions often produce less vigorous reactions, the incidence of apparent disease, characterized by a nasal discharge, being 97 and 53 per cent, respectively. The apparent disease is similarly characterized by a long period of incubation and a tendency toward chronicity.

FROM THE AUTHOR'S SUMMARIES.

COMPARATIVE VALUES OF CLINICAL AND POSTMORTEM BLOOD CULTURES. C. G. BURN and D. F. HARVEY, J. Infect. Dis. **59**:296, 1936.

Of clinical and postmortem blood cultures obtained from 212 persons, 147 (70 per cent) showed growth and 65 (30 per cent) were negative. Agreement between the clinical and the postmortem cultures occurred in 125 (59 per cent) and disagreed in 87 (41 per cent) of the 212 cases. Contaminants were responsible for the discrepancies in 22 (10 per cent) of the cases; this contamination occurred clinically in 18 and post mortem in 4. The greatest discrepancy was due to the presence of negative cultures in 73 (34 per cent) of the cases; in 65 of these it was the clinical cultures, and in 8, the postmortem cultures, that were negative. The most important factor explaining the negative clinical cultures was the time at which the last clinical culture was taken in relation to the death of the patient. Other influences, such as those controlling bacterial invasion into the blood stream, the development of mixed infections in the tissues, and injuries to mucosal linings permitting transient bacteremias caused by invasion from bacterial reservoirs, were important contributory factors in some of these differences. The administration of specific immune serum, the phenomenon of bacterial dissociation and errors in the technic of culturing played a definite but minor rôle in these differences. Postmortem autolysis of the tissues tends to destroy the more sensitive pathogenic bacteria, provided the interval between death and postmortem examination is more than twelve hours.

FROM THE AUTHORS' SUMMARY.

THE PATHOGENESIS AND FATE OF TUBERCLES PRODUCED BY DISSOCIATED VARIANTS OF TUBERCLE BACILLI. W. H. OATWAY JR. and W. STEENKEN JR., J. Infect. Dis. **59**:306, 1936.

A human and an avian strain of tubercle bacilli have been dissociated into variants with widely separated levels of virulence and with distinctive colony forms. The importance of obtaining a complete dissociation by use of the assortment technic and by control of the environment, especially the p_H of the culture medium, has been discussed. The qualities of the variants seem to depend on the bacillary composition.

Three species of animals were inoculated with various quantities of these variants through several portals, and the pathogenesis of the disease was studied. The virulence of the variants was found to be related constantly to the type of colonies in the cultures made before inoculation and after recovery from lesions. Virulence seemed to be more important than the other factors which are known to influence pathogenesis in experimental tuberculosis. It was directly and regularly correlated with the speed and intensity of the cellular reaction and with the progress and fate of tubercle formation.

FROM THE AUTHORS' SUMMARY.

THE DIFFERENT FORMS OF CORYNEBACTERIUM DIPHTHERIAE AND THEIR SIGNIFICANCE. W. MAIR, J. Path. & Bact. **42**:635, 1936.

The "barred" or "intermediate" forms of Corynebacterium diphtheriae all belong to one serologic type. More than 80 per cent of the strains of C. diphtheriae found in definite cases of diphtheria in the London area are of one or the other of two serologic types: the barred form (F) or the starch-fermenting type (A). Forty-five per cent of the strains of C. diphtheriae found in convalescents and

carriers and in persons with conditions the clinical diagnosis of which is doubtful are of the mitis type. Practically all the nontoxigenic strains of C. diphtheriae belong to the mitis type. The different forms of C. diphtheriae may change in artificial culture from one to another. FROM THE AUTHOR'S SUMMARY.

ACTINOMYCOSIS OF THE OVARY. F. H. CÔTÉ and G. R. TUDHOPE, J. Path. & Bact. **42**:673, 1936.

Four cases of actinomycosis of the ovary are reported. The lesion presents to the naked eye the typical characters of the disease as it occurs in other parenchymatous organs, e. g., the liver and kidney, and is diagnosable as soon as the ovary is sliced, if not before. In each case the right ovary was first affected, and in three the history suggests that the appendix was probably the primary focus of infection. FROM THE AUTHORS' SUMMARY.

Immunology

THE SENSITIZATION OF ANIMALS WITH SIMPLE CHEMICAL COMPOUNDS. K. LANDSTEINER and J. JACOBS, J. Exper. Med. **64**:625, 1936.

In continuation of previous work, experiments in sensitization have been made with various substances such as urushiol, benzyl chlorides and acyl chlorides. In the case of a series of substituted benzenes (Cl, NO_2) a connection between sensitizing capacity and lability of the Cl or NO_2 groups has been shown, indicating the formation of conjugated antigens in the animal. This led to the study of benzyl and acyl chlorides, which were found to have sensitizing capacity. Most informative as to the relationship between reactions of the skin surface and anaphylaxis were experiments with acyl chlorides. Guinea-pigs sensitized with p-chlorobenzoyl chloride showed, on one hand, the usual surface lesions after application of the substance, and, on the other, typical anaphylactic shock following intravenous injection of a compound of p-chlorobenzoyl chloride and guinea-pig serum. From this it may be inferred that the two types of allergic manifestation are closely related. FROM THE AUTHORS' SUMMARY.

THE RELATION BETWEEN ANTIANAPHYLAXIS AND ANTIBODY BALANCE. M. C. MORRIS, J. Exper. Med. **64**:641 and 657, 1936.

Sensitized guinea-pigs treated by injection of normal rabbit or guinea-pig serum previous to intravenous inoculation of antigen may be protected against a few lethal doses of antigen. The protection is greater with foreign than with homologous serum and appears to be related roughly to the amount of serum introduced. Sensitized guinea-pigs given injections of antibody-containing serum preliminary to intravenous injection of antigen show no greater refractoriness to anaphylaxis than to those given injections of normal serum. Moreover, in many instances, the injection of an excess of antibody into the circulation of sensitized guinea-pigs leads to increased susceptibility to anaphylaxis. These results indicate that an excess of circulating antibody is not responsible for a state of antianaphylaxis but, on the contrary, may contribute toward the anaphylactic reaction.

Guinea-pigs passively sensitized with antihorse or antipneumococcus serum and specifically desensitized do not manifest as great a reactivity on resensitization with the same antiserum as on the original sensitization. Guinea-pigs passively sensitized with anti-Friedländer type B serum or antipneumococcus type II serum and specifically desensitized do not attain the same degree of reactivity as normal animals when passively sensitized with antihorse serum. Guinea-pigs passively sensitized with anti-Friedländer type B serum and desensitized with the specific carbohydrate remain as resistant to infection with Friedländer's bacillus type B as undesensitized guinea-pigs. Since in this case, at least, it is agreed that type-

specific immunity and type-specific hypersensitiveness are due to the same type-specific antibody, a change in anaphylactic response should be accompanied by a change in immune response, provided this change depends on antibody balance. A determination of the antibody content of the serum of sensitized as well as of desensitized guinea-pigs by mouse protection tests indicates that loss of reactivity in desensitized animals cannot be adequately accounted for on the basis of depletion of circulating antibody. These experiments suggest that hypersensitiveness and resistance are different manifestations of the same antigen-antibody reaction while antianaphylaxis is a state of refractoriness which is due neither to excess of circulating antibody nor to depletion of antibody but is the result of secondary changes, the true nature of which is still not definitely established.

FROM THE AUTHOR'S SUMMARIES.

ISO-ANTIGENS IN SALIVA. G. A. MATSON and E. O. BRADY, J. Immunol. **30**:445, 1936.

Matson and Brady confirm the observation that the blood group substances (including the O) are in the saliva of some persons, according to the mendelian laws of inheritance. The saliva itself, and not accidental admixtures, is the carrier of the group-specific properties. The ferment that is known to destroy the blood group substance occurs in the saliva of some persons independently of the presence or absence of the blood group substance.

I. DAVIDSOHN.

BLOOD GROUPING OF MUMMIES. G. A. MATSON, J. Immunol. **30**:459, 1936.

By use of the method of absorbing the iso-agglutinins from an O serum and the hetero-agglutinins (anti-O) from a treated bovine serum with saline extracts of repeatedly frozen and thawed tissues, the blood groups of Indian and of Egyptian mummies were determined. The Indian mummies (sixteen in all) belonged to group O, while two of the six Egyptian mummies contained the group property B, and one, the property A.

I. DAVIDSOHN.

ANAPHYLAXIS IN MACACUS RHESUS. N. KOPELOFF, L. M. DAVIDOFF and L. M. KOPELOFF, J. Immunol. **30**:477, 1936.

Contrary to previous reports about the difficulty of producing anaphylaxis in the lower monkeys, Kopeloff and his associates encountered no such difficulty in the rhesus money, injecting egg white as the sensitizing antigen by the intravenous route; in one instance sensitization was successfully effected through the gastro-intestinal tract, and in another, by local application to the surface of the brain. A single large dose (10 cc.) was sufficient to cause sensitization. An attempt to produce passive anaphylaxis was successfully carried out in one monkey by injecting into it antiegg-white serum of a rabbit and then, eighteen hours later, 10 cc. of egg white. The observations at autopsy differed greatly from those in anaphylactic shock in the lower animals; they were marked dilatation of all the chambers of the heart, gastric distention and some congestion of the liver. A picture like the Arthus phenomenon was observed in the brains of the sensitized monkeys, characterized by necrosis; the clinical symptoms corresponded with the location of the lesion. No precipitin was detected in the circulation of the animals that exhibited the phenomenon of local cerebral anaphylaxis.

I. DAVIDSOHN.

THE RETICULO-ENDOTHELIAL SYSTEM AND ANAPHYLAXIS IN THE DOG. M. A. MILLS and C. A. DRAGSTEDT, J. Immunol. **31**:1, 1936.

Dogs were sensitized to horse serum. They were then given intravenous injections of india ink or of saccharated iron oxide prior to intravenous injections of test doses of horse serum. The blockade of the reticulo-endothelial system

was apparent from the retention of bromsulphalein, but the usual anaphylactic reaction was not influenced. In two experiments the blockade was effected before the sensitizing injections without interfering with the sensitization.

I. DAVIDSOHN.

THE PERMEABILITY OF THE LUNGS TO ANTIBODIES. J. P. FOX, J. Immunol. **31**:7, 1936.

Intratracheal injections (*a*) of homologous antiserums against typhoid and paratyphoid B bacilli into rabbits and of such rabbit antiserums into dogs, (*b*) of antisheep hemolytic serums into rabbits and (*c*) of type I pneumococcus antiserums into rabbits showed only very slight permeability of the lungs to such antibodies. Therefore, the lungs are not a suitable route for general passive immunization. On the other hand, the retention of these antibodies in the pulmonary parenchyma for considerable periods justifies the use of this method when local accumulation of antibodies in the lung is advantageous.

I. DAVIDSOHN.

GROUP-SPECIFIC AGGLUTININS FOR HUMAN CELLS IN RABBIT SERUMS. C. A. STUART and others, J. Immunol. **31**:25 and 31, 1936.

A study of 422 more or less inbred rabbits showed group-specific anti-A agglutinin in 39 per cent, anti-B agglutinin in 16 per cent and absence of any agglutinin for human red blood cells in 38 per cent. Of the serums, 21 per cent agglutinated only A erythrocytes and 3 per cent only B erythrocytes. Marked agglutination of O red cells was found only in the serums of rabbits from 3 to 5 months old. According to the author, the anti-A agglutinin in the blood serum of the rabbit is a mendelian recessive character. Less definite is the evidence in favor of a similar behavior of the anti-B agglutinin.

Injection of A blood cells into 19 rabbits with normal anti-A agglutinin was followed by development of group-specific anti-A agglutinin to high titers, while no such specific response occurred in 22 rabbits without normal anti-A agglutinin. It is concluded that the presence of normal anti-A agglutinin is essential for the production of group-specific anti-A agglutinin.

I. DAVIDSOHN.

ACTIVE IMMUNIZATION AGAINST POLIOMYELITIS. S. D. KRAMER, A. E. SOBEL, L. H. GROSSMAN and B. HOSKWITH, J. Immunol. **31**:167, 183, 191 and 199, 1936.

It was the authors' purpose in this investigation to test the claims of Brodie that he had obtained efficient active immunization of monkeys and children with his formaldehydized vaccine. Brodie's technic was faithfully followed: The immunizing dose was 5 cc. of a 10 per cent suspension of the virus (corresponding to 0.5 Gm. of cord) rendered noninfective with solution of formaldehylde (1:1,000). The thirty-two monkeys treated failed to show any evidence of immunity. Of fourteen treated children, 50 per cent showed the neutralizing substance, while of seventeen nonvaccinated controls, 41 per cent showed evidences of immunity. A difference of 9 per cent in favor of the vaccinated group did not appear significant. A recheck of Kolmer's method of active immunization against poliomyelitis with five intracutaneous and subcutaneous inoculations of 0.1 cc. per kilogram of a 4 per cent suspension of poliomyelitic cord in 1 per cent sodium ricinoleate at intervals of from five to seven days revealed the failure of the sodium ricinoleate to cause attenuation and the failure of the recommended procedure to protect more than two of the thirty monkeys against intracerebral inoculation of the virus, although in sixteen of the animals sufficient neutralizing substance developed in the blood to neutralize a single constantly infective dose of the virus. The virus was neutralized with convalescent serum, and the mixture was inoculated into monkeys intracutaneously and subcutaneously, with moderate degrees of partial immunity to

intracerebral inoculation of the virus resulting. The degree of immunity was not comparable to that resulting from a severe attack of the disease. The neutralized mixture proved noninfective on subcutaneous and on intracerebral inoculation. Subcutaneous inoculations of the equivalent of from 1 to 2 Gm. of purified virus adsorbed on aluminum hydroxide gel produced in a large proportion of monkeys a solid immunity to intracerebral inoculation of the virus. None of the animals gave any evidence of illness.

I. DAVIDSOHN.

SEROLOGIC CLASSIFICATION OF THE BRUCELLA GROUP. L. VEAZIE and K. F. MEYER, J. Infect. Dis. **58**:280, 1936.

By means of monospecific serums over 400 strains from twenty different countries have been classified. All the smooth cultures were easily separated into two main types, the serologic "abortus-suis" and "melitensis" types. A small subtype was observed, consisting of 26 strains, which is similar to the abortus type in antigenic make-up. It is differentiated from the true abortus type by a greater proportion of melitensis antigen.

A comparison of the serologic findings with those obtained by means of dye reactions and of hydrogen sulfide production revealed conflict in the results in only 5.8 per cent of the tests. All the cultures classified as "suis" by biochemical reactions fall into the abortus group antigenically. Ten per cent of the cultures identified as "bovis" by dye reactions are serologically indistinguishable from "melitensis" cultures. Forty-two per cent of these strains were isolated from a single herd of cattle in the United States. Their reactions indicate the value of systematic classification in epidemiologic studies. It is suggested that certain investigators who have obtained unsatisfactory results from serologic studies have encountered an undue proportion of such strains. Twelve per cent of the cultures which are biochemically "melitensis" are serologically identical with the abortus type. All of these are old laboratory strains of unknown origin (Austria, Italy, Africa and Tunis).

The serologic classification of the strains of Brucella is an indispensable part of the proper identification of the strains for epidemiologic purposes. Its failure to agree with other methods of differentiation in a certain percentage of cases serves to emphasize the fallacy of relying on any one test for proper and complete identification.

FROM THE AUTHORS' SUMMARY.

THE WEIL-FELIX REACTION OF THE RABBIT IN THE DIAGNOSIS OF ROCKY MOUNTAIN SPOTTED FEVER (EASTERN TYPE). K. F. MAXCY, J. Infect. Dis. **58**: 288, 1936.

The direct inoculation of a rabbit with citrated blood obtained from a patient early in the course of the illness and subsequent observation of this animal for the appearance of specific proteus OX agglutinins afford a simple and useful procedure in confirming the diagnosis of Rocky Mountain spotted fever (eastern type). It is particularly valuable if a fatal outcome would prevent these observations being made on the blood serum of the patient.

FROM THE AUTHOR'S SUMMARY.

THE SEROLOGIC TYPES AMONG GRAVIS STRAINS OF CORYNEBACTERIUM DIPHTHERIÆ AND THEIR DISTRIBUTION. D. T. ROBINSON and A. L. P. PEENEY, J. Path. & Bact. **43**:403, 1936.

A technic for the preparation of gravis suspension is described which can be used as a routine procedure. By means of the agglutination test 739 starch-fermenting (gravis) strains derived from many different parts of the world have been examined and have fallen into five types, which are shown to be distinct by the agglutinin absorption test. Types I and II show the classic cultural characters first described by McLeod. Types III, IV and V frequently differ in form of colony and growth in broth from that portrayed in the classic description and, in

these characters, may approach the mitis type. There are no constant features of differentiation between types III, IV and V. Type II has an almost world-wide distribution. Type I is much more restricted but appears to be predominant in Great Britain. Type III is rare outside Great Britain. Type IV is the only type so far discovered in Egypt; it also occurs rarely in Great Britain. Type V has been found only in the United States of America. We are unable to state whether there is any special correlation between serologic type and clinical severity, but types I, II and III at least are known to cause severe infection of epidemic distribution. We have been unable to detect any difference in the serologic characters of organisms from convalescents, carriers and contacts; repeated swabs reveal the same serologic type. Organisms may vary in their colony form and may lose their starch-splitting capacity without changing their serologic properties. Antigenic structure appears to be the most stable bacterial character. Serology does not provide a means of distinguishing between virulent and avirulent strains.

FROM THE AUTHORS' SUMMARY.

THE PRODUCTION OF REVERSED ANAPHYLAXIS IN MAN. C. E. KELLETT, J. Path. & Bact. **43**:503, 1936.

Experimental evidence is submitted which appears to show that in man a phenomenon known as reversed anaphylaxis may occur. It appears that this is the mechanism underlying the local responses which occur in man following the intradermal injection of horse serum. It is suggested that this is the mechanism responsible for the occurrence of serum disease in many, and it is pointed out that many of the known clinical features of this condition are in accord with this hypothesis. It is tentatively suggested that such a mechanism may play an important rôle in the production and localization of many of the so-called allergic responses in man, and it is pointed out that, should this hypothesis prove to be correct, fresh avenues of treatment would thereby be opened up.

FROM THE AUTHOR'S SUMMARY.

THE AGGLUTINOGEN IN THE RED BLOOD CELLS OF THE RABBIT AND ITS RELATION TO THE BLOOD GROUP B. M. EISLER and A. HOWARD, Ztschr. f. Immunitätsforsch. u. exper. Therap. **87**:184, 1936.

The anti-B iso-agglutinin of human A serum was absorbed by rabbit red cells as effectively as by human B blood. This statement is contrary to some previous reports. Human A serum contains the anti-B iso-agglutinin and the antirabbit hetero-agglutinin; human B serum contains no anti-B iso-agglutinin but only anti-rabbit hetero-agglutinin. Quantitative determinations established the presence of antirabbit hetero-agglutinins in higher titer in persons of group A than in those of group B. The human blood group factor B consists of two fractions; only one of them is shared by the red cells of the rabbit. I. DAVIDSOHN.

INTERRELATIONS BETWEEN IMMUNOLOGIC AND ALLERGIC MANIFESTATIONS. E. ROTH and N. VON SZENT-GYÖRGYI, Ztschr. f. Immunitätsforsch, u. exper. Therap. **87**:215, 1936.

Rabbits were sensitized with horse serum and inoculated with suspensions of killed typhoid bacilli. A decrease of the formation of agglutinin was noted if the procedures were carried out at the same time, but no such effect was found if the sensitization preceded the inoculation of the bacilli. The agglutinin response was particularly poor if the liver was damaged by administering phosphorus prior to the simultaneous inoculation and sensitization, but if the phosphorus poisoning was produced after the inoculation of the bacteria, the agglutinin titer remained unchanged. According to the authors, when resistance rises, anaphylactic susceptibility is lessened and vice versa. I. DAVIDSOHN.

Tumors

METASTASES OF INTRACRANIAL TUMORS. A. A. NELSON, Am. J. Cancer **28**:1, 1936.

A case of medulloblastoma of the cerebellum with discrete metastases to the vertebral bodies is reported. The reported cases of metastasis of an intracranial tumor are reviewed.

FROM THE AUTHOR'S SUMMARY.

VAGINAL METASTASES FROM HYPERNEPHROMA. J. SHARNOFF and A. M. SALA, Am. J. Cancer **28**:20, 1936.

Four cases of hypernephroma with vaginal metastases are reported. Reports of sixteen cases in the literature are reviewed. The various theoretical explanations of this occurrence are discussed. The most probable explanation seems to be that the vaginal growths represent implantation metastasis by way of the urinary tract.

FROM THE AUTHORS' SUMMARY.

NEOPLASTIC DISEASES PRODUCED IN MICE BY GENERAL IRRADIATION WITH X-RAYS. J. FURTH and O. B. FURTH, Am. J. Cancer **28**:54, 1936.

Mice were exposed to a single dose or repeated doses of roentgen rays at the age of approximately 5 to 12 weeks; 775 mice were irradiated, and 1,290 were kept as controls. Ovarian tumors were fifteen times as frequent in the irradiated mice as in the controls; myelosis was eight times as frequent and mediastinal lymphosarcoma seven times as frequent in the irradiated mice as in the controls. There was a slight but definite rise in the incidence of several other neoplasms, but the incidence of cancer of the breast decreased by one half. The occurrence of cancer of the breast appears to be conditioned by activity of ovarian hormone, which is decreased by irradiation, though in the irradiated mice it apparently increased with the development of the ovarian new growths. The high incidence of cancer of the breast with ovarian neoplasms and the associated endometrial hyperplasia suggest that some ovarian tumors produce hormone. The roentgen ray-induced tumors were indistinguishable from those occurring spontaneously and appeared at a somewhat earlier age. It is suggested that roentgen rays alter the constitution of certain cells so that they or some of their offspring undergo malignant transformation several months after irradiation.

FROM THE AUTHORS' SUMMARY.

NEOPLASTIC DISEASES AMONG MICE SUBJECTED TO GENERAL IRRADIATION WITH X-RAYS. J. FURTH and J. S. BUTTERWORTH, Am. J. Cancer **28**:66, 1936.

The destructive changes in the ovaries of mice which immediately follow irradiation with roentgen rays are followed by slowly progressive proliferative changes, resulting after from one to two years in the formation of growths with the character of neoplasms. All of the solid ovarian tumors found have occurred in senile mice. Tubular adenomas arise from downgrowth of germinal epithelium in the form of epithelial canals. There is no evidence that these growths have endocrine function. Proliferation of follicular epithelial and interfollicular spindle-shaped cells, both of which are probably derivatives of germinal epithelium, gives rise to granulosa cell tumors. These tumors may be composed of spindle-shaped cells like those of the ovarian stroma, of epithelial cells like those of ovarian follicles, of luteinized epithelial cells or of several different morphologic variants of the granulosa cells. In many of the mice with granulosa cell tumors excessive endocrine function is indicated by extensive cystic glandular hyperplasia of the endometrium; in a few mice, by hyperplasia of the endometrium, and in a few mice, by hyperplasia of the anterior lobe of the hypophysis. The incidence of mammary tumors is greater among mice with granulosa cell tumors than among unirradiated females of the same stock but is less among irradiated mice with no

ovarian tumor or with tubular adenoma of the ovary. Sterilization of the ovaries, however, does not cause cessation of the production of estrin and does not altogether prevent the formation of mammary tumors.

FROM THE AUTHORS' SUMMARY.

COAL SMOKE SOOT AND PULMONARY TUMORS IN MICE. M. G. SEELIG and E. L. BENIGNUS, Am. J. Cancer **28**:96, 1936.

Of mice exposed to soot over a long period, 8 per cent acquired carcinoma of the lung in contrast to 2 per cent in a colony not subjected to soot. There is a greater incidence of cancer of the lung among human beings in cities than among those living in the country. While it is impossible to establish offhand a direct causal relationship between smoke and soot and cancer of the lung in man, census statistics are suggestive enough to stimulate further studies of the subject.

FROM THE AUTHORS' SUMMARY.

SUCCESSFUL TRANSPLANTATION OF A HEPATOMA IN MICE. L. C. STRONG and G. M. SMITH, Am. J. Cancer **28**:112, 1936.

A hepatoma developing spontaneously in a mouse of the CBA strain was successfully grafted into relatives of the mouse. The grafted cells grew and remained physiologically active (in the secretion of bile) at least five months after the tissue was implanted subcutaneously into these other mice. The grafts showed a higher incidence of mitotic figures than the original tumor. Neither the original hepatoma nor the grafted tumors appeared to have biliary duct systems. The tumor is favorable material for cytologic investigations.

FROM THE AUTHORS' SUMMARY.

THE VIRUS TUMORS AND THE TUMOR PROBLEM. P. ROUS, Am. J. Cancer **28**:233, 1936.

"What real difficulties stand in the way of the supposition, for experimental purposes, that the general run of malignant growths are due to viruses? They can be assembled, some of them from books, some out of one's own thought, and they are best discussed in one-two-three order so as not to get lost among them.

"(1) The world-wide occurrence of cancer: It is plain that the cause of cancer must be present wherever man is. But wherever he goes so do certain of his parasites. He takes with him his colon bacilli and his lice. May he not take viruses as well?

"(2) The sporadic occurrence of cancers as attesting to lack of infectiousness: Tumors are highly conditioned diseases, whatever their nature, dependent upon a concatenation of factors—heredity, age, chronic irritation, etc., etc. The more a disease-producing agent is conditioned in its activity, the less will the evidence be, until there is none, that it is infectious in character. The natural incidence of the chicken tumors yields no sign whatever that they are caused by a virus. Their occurrence is highly conditioned and some of them obviously represent a triumph over resistance offered by the host. If all mice were tarred, thus rendering them favorable, mouse cancer would be pandemic.

"(3) The failure of attempts to demonstrate an extrinsic cause for the generality of malignant mammalian tumors: Frequently virus cannot be obtained from chicken tumors known to be due to it, and often the Shope virus cannot be got again from the papillomas of cottontail rabbits, much less from those of domestic animals. In stressing the negative results with mammalian cancers, have technical difficulties been mistaken for a biological principle?

"(4) The hereditary determination of tumors: Tuberculosis was deemed hereditary before the bacillus was recognized. The appearance of malignant

tumors of the same sort in identical twins, in cases of hereditary glioma of the retina, and of von Recklinghausen's disease, may mean no more than that when the soil is right, and the contributory circumstances, a carcinogenic agent, perhaps a virus, is effective as it would not otherwise be.

"(5) The experimental induction of cancer at sites where it never normally occurs: The tumors that have given rise to this difficulty, those, for example, which result from tarring the ears of laboratory animals, are not in the real sense tumors induced at will. Their incidence varies notably from individual to individual; they occur at relatively few places in large areas subjected to carcinogenic stimulation; they are punctate in origin; and though in any one individual their number may increase as tarring or other stimulation is continued, no experimental procedure thus far employed has caused them to appear as diffuse processes or in unexampled multitudes. Some decisive condition or agent is evidently present at the situation where they arise. Andrewes has given reasons for supposing this agent to be a virus entering the organism previously, and ensconced in the epithelium at the time when the carcinogenic substance is applied —an indigenous virus as he terms it.

"(6) Cancer does not spring full-blown from normal cells but develops as the result of gradual and often long-continued changes: The changes induced by all the various carcinogenic agents may be of a sort to urge a symbiotic virus or viruses to pathogenic activity. A mere dietary error will bring out a crop of fever blisters on the skin of a man in whom herpes virus has lain latent. The virus of lymphocytic choriomeningitis, which exists in the brains of mice that are to all appearances normal, will become active and kill if a little bouillon is injected intracerebrally. By injecting Scharlach R into virus-induced rabbit papillomas they can be made to keep on growing while untreated growths are retrogressing in the same animal.

"(7)) Metastases of several differing sorts, representative of more than one germ layer, are occasionally encountered in patients dying of a teratoma that becomes malignant: Many teratomas are supposedly derived from pluripotential sex cells, and if one of these became infected with a tumor-producing virus secondary growths of diverse character would occur as a matter of course.

"(8) An enormous variety of malignant tumors exists, deriving as they do from cells of nearly all kinds, and exploiting the wide capabilities of these cells: It is urged that since viruses are highly specific in their action, one causing osteochondrosarcomas of the fowl, for example, another endotheliomas only, an entire microcosm of viruses would be needed to account for all the malignant tumors.

"This is an a priori objection and the future can be left to take care of it. Medical workers are now beginning to realize through studies of herpes, and submaxillary gland virus, the virus causing lymphocytic choriomeningitis, virus III and others, that the healthy body may have a virus population comparable with that of bacteria but far more considerable and diverse. For whereas bacteria are forced by the body defenses to live literally in holes and corners of the organism, upon stretches of mucous membrane or in pockets at situations where antibodies and leukocytes do not get at them, viruses are protected by the very cells that they infect, unless they kill these and thus expose themselves to neutralization by serum and to attack of other sorts. Wherever a cell is, there may a virus live, if symbiosis is enough for its needs or if it merely causes the cell to divide and to go on dividing. The variety of the cells is legion, and life in association with them should infallibly lead to great specialization—just such as exists in the case of the chicken tumor viruses and that causing the rabbit papilloma. Yet the viruses of some chicken tumors have a group relationship, as Andrewes has shown, and a single virus of this sort may give rise to tumors that vary not a little within their type. Whatever the cause for the rabbit cancers, it acts only upon epidermal cells, and produces changes in these that take a special direction; yet the variety of the resulting tumors—cystic tumors, malignant papillomas, squamous-cell carcinomas—is not inconsiderable. The theoretical need for a vast multiplicity of viruses is lessened by such findings.

"These are the main factual obstacles to the view that sarcomas and carcinomas can be caused by viruses; and they fade as one looks closely at them.

"How far should one be led by the assumption that certain tumors may be due to viruses? Only so far as to make tests with these growths. The tumor problem has withstood the most corrosive reasoning. Yet since what one thinks determines what one does in cancer research, as in all else, it is well to think something. And it may prove worth while to think that one or more tumors of unknown cause are due to viruses."

EFFECTS OF OSMOTIC PRESSURE ON NORMAL AND MALIGNANT FIBROBLASTS AND THE PERMEABILITY OF NORMAL AND MALIGNANT CELLS TO WATER. A. M. BRUES and C. M. MASTERS, Am. J. Cancer **28**:314 and 324, 1936.

By estimations of the volumes of fibroblasts made from areal dimensions of cultured cells undeformed by environment, the effects on these cells of changes in osmotic pressure have been determined. The increased volumes which resulted from diminished pressure showed no apparent differences between embryonic and sarcomatous cells. As to "osmotically inactive" material, this was as a rule slightly greater in malignant cells—an average of 26 as compared with 22 per cent—but there were wide individual variations. In regard to migration and growth, sarcoma cells (mouse sarcoma 180) were more greatly, and adversely, affected by osmotic changes in either direction than were embryonic chicken cells.

An attempt was made to determine the rates of absorption of water by various cells by making similar measurements and taking into account the rates of diffusion of salts from clotted plasma into fluids of lower concentration, as determined by the use of erythrocytes as osmometers. The curves of volume changes so obtained were matched against theoretical curves constructed with various permeability constants, and their constants so obtained. The results gave permeability constants of rat and chick embryo heart, mouse sarcoma 180 and Walker rat tumor 256 which ranged from 0.4 to 1—figures between those of the egg of Arbacia and the erythrocyte. There was no significant divergence between the several groups of cells. Brues and Masters were unable to establish any significant differences in permeability between avian and mammalian cells or between embryonic and sarcomatous fibroblasts.

H. E. EGGERS.

THREE HUNDRED MIXED TUMORS OF THE SALIVARY GLANDS, OF WHICH SIXTY-NINE RECURRED. JOSEPH MCFARLAND, Surg., Gynec. & Obst. **63**:457, 1936.

This is the fourth of an unnumbered series of studies on tumors of the salivary glands extending over a period of ten years, each study embracing a larger series of tumors than its predecessors. So far as possible the patients from whom the growths were removed were followed over a period of years in an effort to determine the outcome in each case. Slightly more than 23 per cent of the neoplasms recurred, and 13 per cent proved fatal. After long study, McFarland is convinced that attempts to correlate the histologic structure of mixed tumors of the salivary glands with their clinical behavior have totally failed and that microscopic examination as an aid to prognosis is of no value. Recurrence may take place at any time between the excision of the tumor and the end of a period of forty-seven years; thus it is impossible to prove that any tumor of this type will not have a recurrence. Available data indicate that roentgen ray and radium therapy are not more efficacious than surgical intervention. The importance of postponing operation until the tumors are "ripe," i. e., about the size of a lemon, is emphasized by convincing figures. As a rule, it may be said that the smaller the size of the extirpated growth the greater is the probability of its recurrence.

WARREN C. HUNTER.

Society Transactions

CHICAGO PATHOLOGICAL SOCIETY

Regular Monthly Meeting, April 12, 1937

CARL W. APFELBACH, *President*

EDWIN F. HIRSCH, *Secretary*

CONCEPTION, CONSTITUTION AND SUSCEPTIBILITY TO DISEASE. W. F. PETERSEN.

"Wherefore it is natural to realize that generation too varies in the coagulation of the seed (development of the embryo), and is not the same for the same seed in summer as in winter nor in rain as in drought. It is for this reason, I think that the physique of Europeans varies in each city. For there arise more corruptions in the coagulation of the seed when the changes of the seasons are frequent than when they are similar or alike."—From HIPPOCRATES: *Airs, Water, Places,* paragraph 23.

Today three divergent lines of scientific approach apparently converge to illuminate the ancient observation. In the first place the study of embryology has led to the recognition that in the earliest stages of development of the ovum after fertilization the rapidly dividing cells need proportionately enormous amounts of oxygen and that any interference may be portentous in either stimulating or injuring the young embryo and interfering with its developmental trends. In the second place, the development of knowledge of the genetic mechanisms has led to the recognition that in the early stages of development the gene train may be more susceptible to environmental changes. In the third information has developed revealing the close chemical, endocrine and nervous integration of the human organism with the environment and, particularly, the reflection of this in the reproductive organs—the uterus in the female and the testicle in the male. While the chemical equilibrium is constantly undergoing a rhythm—constantly balancing and integrating the environmental thrusts in an endeavor to maintain the normal balance—at certain times the chemical, endocrine and nervous balance is pushed back more violently. These peaks and depressions are reflected in the oxidative opportunity in the mucous membranes of the uterus and represent at certain times of the year wide variations in the oxidative potential of the embryo. This may result in (1) relative quiescence of the embryonic tissues, (2) absolutely normal conditions, (3) slight hunger for oxygen, with the possibility of maximal stimulation and potentiation, and finally (4) inadequacy of oxygen to such a degree that injury may follow.

In addition, the embryonic metabolism may assume the pace laid down by the maternal organism at the time of early embryonic development, and the possibility must be considered that this pace, once laid down, may be retained in post-embryonic life. In other words, seasonal instability, which reaches its crest in the late winter and spring and which finds its reflection in maternal activation, with endocrine and chemical instability, may be followed by the establishment of a similar trend in the offspring. On the other hand, the seasonal stability that one associates with environmental conditions in the summer and autumn, which finds its reflection in metabolic quiescence in the maternal organism, may establish a similar trend in the offspring conceived and developing during this period. The period of relative instability, of greater environmental and maternal variability, makes probable the creation of more unusual types and possibly of more slender types in the late winter and spring, with a trend toward greater broadness and toward more normal types in the later summer and autumn. One also knows

from long medical and pathologic experience that certain diseases are associated with certain types of body build. This holds true not only for mental disease but for diseases such as diabetes, heart disease, kidney disease, goiter and gastric ulcer; of course, the association of habitus and tuberculosis is a matter of ancient knowledge. If it is true that the trend of the child's metabolism may be established by the trend of the maternal metabolism during early embryonic development, and if it is possible that the child's habitus is conditioned by the maternal environment during early embryonic development, the kind of disease from which the adult may die may in some measure be predetermined in embryonic life, an idea that is again clearly stated in the Hippocratic texts.

This possibility has been studied by examining death certificates of persons who died from different diseases, noting the months of conception of such persons and then computing the excess or the deficiency for each of the different diseases on the basis of the normal conception expectancy for the United States and for the state of Illinois. Some 73,000 records have been examined from this point of view. It was observed, for instance, that there is a greater tendency for persons who die from erysipelas to have been conceived in the first half of the year, while, on the other hand, persons who die from poliomyelitis (infantile paralysis) appear definitely to have been conceived in greater numbers in the summer and autumn. This finding probably means merely that persons of the more slender type die in greater numbers from erysipelas, and persons of the more sturdy type, from poliomyelitis. Prof. George Draper, New York, has already called attention to the fact that it is the stocky, broad type that is more often involved in infantile paralysis. Of persons who died from pulmonary tuberculosis (some 2,000), a somewhat greater number had conceptions in May and June; on the other hand, persons who died from tuberculosis of bones and lymph glands revealed a deficit of conceptions at this time. Persons who died from lobar pneumonia (19,000) revealed an excess of conceptions in the latter part of spring, while persons who died from cerebral hemorrhage showed an excess of conceptions in the summer and autumn. For cancer, some 25,000 death certificates were examined, and the the rate for the total group revealed practically no deviation from the normal expectancy, but there were marked differences for the organs that were involved. Thus persons who died from cancer of the lung revealed a deficit of conceptions in the spring, but women who died from cancer of the genital tract revealed a deficit in the summer.

While provisional, it seems that the old Hippocratic assumption may be demonstrated as apparently correct by modern scientific methods, although it should be definitely understood that a much larger material must be examined before definite statements can be made.

FOREIGN BODY IN THE HEART. BENJAMIN H. NEIMAN.

To the numerous cases of foreign body injury which have been reported in the literature, one of unusual interest is added. A white woman 27 years of age had jumped through a window during a fire. A laceration of the wall of the chest healed without complications. Because of cardiac consciousness, nervousness, fainting spells and pain in the precordial region, she was admitted to the Cook County Hospital six months after the injury. The pericardial effusion was attributed to tuberculosis or trauma. After remaining in the hospital four months, she went home but returned seven months later. She was then about four months pregnant and had severe cardiac decompensation. She went into spontaneous labor five weeks after entrance and delivered a 5 month premature infant, stillborn. During delivery she died. The autopsy revealed a piece of glass, 70 mm. long, from 17 to 25 mm. wide and 2 mm. thick, with six sharp angular corners, lying in the orifice of the tricuspid valve. One end was in the right auricle; the other, in the right ventricle. The myocardium of the right ventricle was scarred, and the cusps of the valve and the leaflets of the pulmonary valve were lacerated.

PRIMARY LYMPHOSARCOMA OF THE LIVER WITH METASTASES TO THE MARROW AND SECONDARY ANEMIA. EDWIN F. HIRSCH.

The article was published in full in the May 1937 issue of the ARCHIVES OF PATHOLOGY, page 674.

BUFFALO PATHOLOGICAL SOCIETY

Regular Meeting, Jan. 24, 1937

KORNEL TERPLAN, *President*

W. F. JACOBS, *Secretary*

ALEUKEMIC RETICULOSIS. STUART VAUGHAN and KORNEL TERPLAN.

About twenty known cases have been reported in the literature under the name of leukemic or aleukemic reticulosis. The case which we present is one in which, clinically, acute leukemia was diagnosed. Only postmortem examination enabled us to make the more specific diagnosis of diffuse reticulum cell hyperplasia involving especially the bone marrow, spleen and lymph nodes but also to some extent the liver and kidneys. The blood findings pointed to complete suppression of the granulated cells and marked anemia. Evidence of excessive destruction of blood was especially seen in the bone marrow and in the spleen. The excessive hyperplasia of the reticulum cells, with many rather mature forms showing a huge cytoplasm and typical nuclear structures, was perhaps an expression of a compensatory effort of the severely damaged bone marrow. An extensive hemorrhagic diathesis and necrotizing lesions in the mouth presented a parallel to the symptomatology of many acute leukemic conditions.

The patient was a white man, 27 years of age, a manual laborer in a chemical factory. He denied that there had been any exposure to, or intimate contact with, a chemical agent. He enjoyed good health until six months previous to death, when he showed increasing weakness, anorexia, loss of weight, headache and migrating pains, especially in the bones of his hands, legs and chest. Four and a half months later, while he still continued to work, he contracted a sore throat with superficial necrosis and shallow ulcers, especially in one tonsil. Two injections of neoarsphenamine were given without any effect. On admission he appeared extremely ill, being very pale with a subicteric tint. The clinical findings included necrotic membranes on the tonsils, base of the tongue and pharyngeal wall, distinctly enlarged cervical and inguinal lymph nodes and a palpable liver and spleen. The temperature was 104.2 F. The red blood cells numbered 1,160,000 per cubic millimeter; the hemoglobin was 40 per cent; the white blood cells numbered 500, and all of these were lymphocytes. The platelet count was 34,800. A blood culture was sterile. Shortly before death a petechial rash appeared on the abdomen.

Post mortem there were found: extensive petechial hemorrhages in the conjunctivae and in the integument, especially that above the chest and abdomen; moderate enlargement of the cervical, axillary and inguinal lymph nodes, which appeared soft and discrete; a soft enlarged liver, 2,600 Gm. in weight; a moderately soft enlarged spleen, 560 Gm. in weight; petechial hemorrhages in the epicardium and in the mucosa of the renal pelves; marked swelling of the kidneys, with distinct anemia, and very marked anemia of the heart. The bone marrow of the ribs and vertebral bodies showed a grayish color, suggestive of myeloid hyperplasia. (The long bones were not examined.)

Histologically, a uniform hyperplasia of smaller and larger reticulum cells was found in all lymph nodes and in the bone marrow, spleen and liver. Many of these, especially those in the bone marrow and spleen, showed massive phagocytosis of red blood cells. The hyperplasia of the reticulum cells in the bone

marrow reached almost blastomatous proportions, leaving only very thin bony trabeculae between these cells. In some areas rather acute regressive changes were seen, with considerable nuclear disintegration and very many so-called naked nuclei. The parenchymatous lesions in the liver and kidneys were most marked and consisted of hyaline globular degeneration of the epithelial cells in the proximal convolutions, of different degrees, and typical zonal necrosis of the liver cells.

CHANGES IN THE INTERVERTEBRAL DISKS IN SPONDYLOLISTHESIS AND RELATED CONDITIONS OF THE SPINE. W. PUTSCHAR.

Gliding displacement of vertebral bodies on each other, brought about by static and dynamic strains and stresses, develops only under special conditions. Either the structural resistance is lowered, as one sees it in typical cases of spondylolisthesis, on the basis of spondylolysis, or the strains to which a certain intervertebral disk is subjected act in an abnormal way. The latter condition is established in cases of kyphoscoliosis in which because of the deformity some disks in the vicinity of the vertex of the curvature meet these static stresses of the lasting burden in a vertical or rather tilted instead of horizontal position. In these cases a lateral rotatory gliding displacement of a vertebral body, especially in the lumbar portion, may occur, as first observed on roentgen films by W. Mueller.

The finer histologic changes in the intervertebral disks, which have not been studied so far, are demonstrated on large sections from one case of spondylolisthesis of the fifth lumbar vertebra in a 60 year old man and from three cases of kyphoscoliosis. The change which allows a progressive gliding displacement of a vertebral body is an almost complete interruption of the annulus fibrosus of the intervertebral disk, leading to the formation of a jointlike cavity with two movable articular surfaces. The beginning stages of this rupture of the disk have been observed in kyphoscoliosis. Progressive gliding displacement as seen in spondylolisthesis causes remarkable damage to the hyaline cartilage on the surface of the vertebral body, which can be demonstrated by the repair through cartilaginous tissue more primitive in character. Compensatory attempts to inhibit further displacement and to establish a new equilibrium can be observed in spondylolisthesis and in kyphoscoliosis. In spondylolisthesis one sees ossification of the anterior part of the involved intervertebral disk, leading to the formation of a stable bony fusion between the balcony-like protruding part of the vertebra and the anterior part of the sacrum. In kyphoscoliosis the rotatory lateral displacement may find compensatory correction in the formation of a marginal exostosis on the adjoining vertebra, as usually observed in spondylosis deformans.

A CASE OF POSTPARTUM AIR EMBOLISM. MARGARET WARWICK.

The patient was a woman 33 years of age who had been delivered of a child five years previously. The present pregnancy and delivery had been entirely normal. She had been put up in knee-chest position for five minutes for three successive days. She was put up again on the fourth, when she suddenly became cyanotic and began to have tonic convulsions. These lasted about thirty minutes, ending in death.

The autopsy showed the heart to contain no clotted or fluid blood but instead a beaten pink froth, which was much more pronounced in the right chambers. Similar frothy blood was found also in the inferior vena cava, both iliac veins and both renal veins. There was a marked emphysema of the lungs.

The uterus was comparatively large and filled with large blood clots. At the site of the placental attachment, there was a rough, dark red area measuring about 5 cm. in diameter, with a large amount of adherent blood clots. Microscopic sections showed no inflammation in the uterine wall.

Apparently there must have been a softening of the endometrium at the former placental attachment. During the knee-chest position, the uterine cavity was ballooned with air, with a stretching of the endometrium and resulting single or

multiple tears, which opened up the sinuses within the wall and allowed air to rush into the venous circulation and through it to the heart. The muscular contractions of the heart beat the blood and air to a froth and prevented circulation of the blood to the lungs.

FOREIGN BODIES IN THE APPENDIX. S. A. ZAWADSKI.

The literature of the past twenty years contains about eighty references to foreign bodies in the appendix. In the Millard Fillmore Hospital there have been nine appendixes containing foreign bodies in a series of 4,011 appendixes removed since 1929—an incidence of 0.18 per cent. Four appendixes contained fruit seeds, three gallstones, one chewing gum, and one a toothpick.

The etiologic relationship of foreign bodies to appendicitis has been much discussed. Foreign bodies are too rare a finding to be considered as the sole cause of appendicitis, but when present they may be the cause or an aggravating factor. They may lead to erosion of the mucosa or to perforation. Foreign bodies may be found incidentally in the appendix as passive matter.

Of the nine appendixes containing foreign bodies found in the aforementioned hospital, in four the foreign bodies were associated with acute appendicitis; in one, with mild acute appendicitis, and in one, with chronic appendicitis; in three there was no inflammatory reaction.

In conclusion, it may be said that foreign bodies in the appendix are rare when one considers the many foreign bodies of different types which pass by the appendical opening on their way down the intestinal tract. Foreign bodies may cause an inflammation of the appendix by injuring the mucous lining, allowing bacteria to invade the wall.

Regular Meeting Feb. 28, 1937

K. TERPLAN, *President*

W. F. JACOBS, *Secretary*

PRODUCTION OF HEMORRHAGIC NECROTIC LESIONS OF THE SKIN BY MEANS OF BACTERIA. ERNEST WITEBSKY and ERWIN NETER.

The development of primary lesions at the sites of infection in chronic infectious diseases has been studied often and thoroughly, especially in syphilis and tuberculosis. The experimental analysis of the primary lesions in acute infections, on the other hand, has been taken up only incompletely.

According to their capacity for producing hemorrhagic necrotic lesions of the skin when injected into the skin of rabbits, three groups of micro-organisms may be differentiated from each other. Haemophilus influenzae, when injected intradermally, produces inflammation and erythema within from twenty-four to forty-eight hours. There is never a hemorrhagic necrotic change to be observed in the involved area of the skin. If, however, the intradermal injection is followed twenty-four hours later by an intravenous injection of H. influenzae (or of the filtrate from the washing of an agar culture, prepared according to Shwartzman's technic), severe hemorrhagic necrotic changes occur in the involved area of the skin from three to five hours after the intravenous injection.

Friedländer's bacillus and Bacillus pyocyaneus, when introduced into the skin of a rabbit, produce inflammation and erythema and sometimes even hemorrhagic necrosis. When heat-killed bacilli are injected intradermally and twenty-four hours later living or heat-killed bacilli are injected intravenously, hemorrhagic necrotic lesions may develop in the previously treated areas of skin.

Pneumococci behave, again, in a different way. Severe hemorrhagic necrotic lesions of the skin may result when pneumococci pathogenic for rabbits are introduced into the skin of a rabbit, followed in most instances by death of the animal.

Blood cultures are always positive. No hemorrhagic necrotic lesions were obtained in preliminary experiments, however, when heat-killed pneumococci were introduced intradermally and twenty-four hours later living or heat-killed pneumococci were given intravenously. If, on the other hand, heat-killed pneumococci are injected into the skin over the abdomen and simultaneously living pneumococci are injected into the skin over the back, both sites may sometimes show marked swelling and erythema, followed by severe hemorrhagic necrotic changes in the involved areas of the skin. The presence of pneumococci in the blood stream is held responsible for the occurrence of hemorrhagic necrotic changes in the pneumococcic infection of the rabbit skin. An overwhelming infection of the blood stream seems, however, to be necessary for the elicitation of a reaction of this type, because pneumococci given intravenously have failed so far to produce a similar lesion.

NATURE AND ORIGIN OF THE HEMORRHAGIC NECROTIC LESIONS OF THE SKIN CAUSED BY BACTERIA AND BACTERIAL PRODUCTS. ERNEST WITEBSKY.

For the explanation of the hemorrhagic necrotic lesions of the skin described in the preceding paper and similar lesions caused by the filtrate from the washing of a bacterial agar culture, prepared according to the Shwartzman technic, the following working hypothesis is made: The bacteria in question, as well as the bacterial products, when injected intradermally, are apparently first absorbed by the endothelial cells of small blood vessels and capillaries, according to histologic examinations carried out by several authors in connection with the Shwartzman phenomenon. In analogy to the significance of opsonic action in phagocytosis, normal antibody functions of the blood serum are considered important for intracellular absorption of the substances which are injected intradermally. (The same mechanism, however, holds true if the first injection is given intravenously instead of intradermally.) Consequently, there is a considerable increase in the concentration of such normal antibodies at the site of the injection.

If the second injection is then given intravenously (or an invasion of the blood stream occurs following infection with certain living pathogenic bacteria), the injected substances are conducted to the area of increased concentration of antibodies. This is the site of the first intradermal injection. Morgenroth described what he called "transgression of amboceptors": If sensitized red cells are mixed with nonsensitized cells, antibodies are separated and may sensitize the nonsensitized normal red cells which were added. That toxin-antitoxin combinations are reversible under certain conditions is an established fact. The sudden appearance of new active substances at the site of the first injection may induce a reaction according to the experimental findings mentioned; i. e., a partial dissociation of the antigen-antibody compound may take place within the cells. The detrimental effect which becomes evident may be explained by the fact that toxic substances, absorbed in a neutralized phase, are liberated and exert their toxic action within the cell. Compared with the action of immune antibodies, certain normal antibody functions of the blood serum are characterized by a distinct lack of specificity. This explains the experimental experience that a wide variety of substances may be used for the second intravenous injection. If this working hypothesis should stimulate further experimental work, I shall feel justified in having presented it.

AN INFECTION OF THE URINARY TRACT WITH DYSENTERY BACILLI. E. NETER and O. HOSTERMAN.

In contradistinction to typhoid fever, intestinal dysentery is rarely characterized by the presence of the specific bacilli in the urine. The following report is concerned with the bacteriologic findings in a case of infection of the urinary tract with dysentery bacilli.

The patient was a girl 2½ years old who was admitted to the Children's Hospital in November 1936 with a history of fever of three days' duration. The

important clinical findings were those of an infection of the urinary tract. A catheterized specimen of the urine revealed albumin (4 plus) and sediment loaded with white blood cells and bacilli. On bacteriologic examination, a nonmotile nonlactose-fermenting bacillus, which grew well on ordinary mediums, was found in pure culture. The strain produced acid but no gas in the butt of a Russell double sugar agar slant, with no change on the surface; it fermented dextrose, maltose and mannitol, with the production of acid, but did not ferment lactose and saccharose. The strain produced indole within twenty-four hours. Agglutination tests yielded observations as follows: A polyvalent dysentery serum agglutinated the strain up to the dilution of 1:320. Of the monovalent dysentery serums, Hiss serum agglutinated up to the dilution of 1:640; Flexner serum, up to the dilution of 1:20. Shiga and Sonne serums were negative in the dilution of 1:10. Typhoid, paratyphoid A and paratyphoid B serums (dilution 1:10) also failed to agglutinate the bacilli. These were to be considered, therefore, as dysentery bacilli of the Hiss type. The serum of the patient agglutinated the strain under discussion up to the dilution of 1:640. On four subsequent occasions, catheterized specimens of the urine were examined, and micro-organisms of the same type were found to be present in pure culture.

The child was treated with ammonium chloride and mandelic acid. After two weeks, the temperature was normal and the clinical symptoms of the infection of the urinary tract had disappeared, and during the third week the urine became sterile.

Although the patient presented no history or clinical evidence of intestinal dysentery, dysentery bacilli of the Hiss type could be constantly recovered from the feces even after the infection of the urinary tract had subsided. Therefore, the child may be considered as a carrier of dysentery bacilli in whom an acute autoinfection of the urinary tract developed.

ACUTE AND CHRONIC POLYMYOSITIS. S. SANES, C. W. GREENE and K. TERPLAN.

Two cases of nonsuppurative polymyositis are presented. In the first patient, the disease assumed an acute form. In the second, the clinical and pathologic observations were those of a chronic stage.

CASE 1.—A 32 year old white man had an area of cellulitis in the right forearm drained. The exudate was not examined bacteriologically. One month later he became ill with what he called the grip. Acute symptoms and fever persisted for four days. Then he was unable to resume work because of mental dulness, sore eyes, nausea, chilly feelings, cold perspiration, difficult breathing, muscular weakness and pain. On admission to the hospital he showed a rash on the forehead. The face was swollen. The tongue was raspberry red. The skin was hyperemic and edematous. The patient could not raise his legs. The temperature was subnormal. The laboratory findings were: 19,600 white blood cells per cubic millimeter, with 89 per cent polymorphonuclears; a sterile blood culture; occult blood in the stool. Death occurred three weeks after onset of the illness.

The pathologic diagnosis was: acute serous hemorrhagic polymyositis; distinct degeneration of the myocardium with a few small hemorrhages; edema of the skin of the chest; fresh ecchymoses in the mucosa of the stomach, colon and rectum; cloudy swelling of the kidneys with hyperemia and old hemorrhage in the tubules; fatty change in the liver; slight acute inflammatory hyperplasia of the lymph nodes in the pelvis and along the aorta and inferior vena cava; acute emphysema of the lungs; a decreased amount of lipoid in the adrenal cortex.

Histologically, the muscle fibers showed marked regressive lesions. They were swollen. The striations had disappeared. Granular vacuolar and fatty degeneration was present. In some places there was separation of myofibrils. Fragmentation had occurred. Interstitial inflammation was slight, with scattered lymphocytic infiltration, In many areas recent interstitial hemorrhage was prominent. Cultures taken from different portions of the acutely diseased muscles did not reveal any pathogenic bacteria.

CASE 2.—A 48 year old Negress became ill with pain in the feet, ankles, knees, hips, shoulders and elbows, which forced her to go to bed. On physical examination three months after the onset of these complaints, the patient was unable to open her mouth or protrude the tongue. She drooled saliva. There was difficulty in swallowing. The left sternomastoid muscle was atrophied. The small muscles of the hands and the muscles of the legs also showed atrophy. The deep reflexes could not be obtained. There was moderate anemia with polymorphonuclear leukocytosis. Two blood cultures were sterile. A low grade fever persisted. The patient died eleven months after the first complaint.

The pathologic diagnosis was: chronic polymyositis with fibrosis and atrophy; ulcerative esophagitis (lower portion); cellulitis of the left side of the face; purulent extradural inflammation; bilateral orbital cellulitis; purulent bronchiolitis and bronchopneumonia with emphysema; fatty change in the liver; slight fibrous endocarditis of the mitral valve; distinct patchy myocardial fibrosis; chronic fibrous contraction of the gallbladder; decubitus over the sacrum.

In addition to recent degenerative changes of muscle fibers, the histologic sections showed predominantly interstitial inflammation with infiltration of round cells and plasma cells, very marked fibrosis and atrophy of muscle fibers. The brain and spinal cord were examined; no lesions were found.

PATHOLOGICAL SOCIETY OF PHILADELPHIA

Feb. 11, 1937

ESMOND R. LONG, *President*

HERBERT L. RATCLIFFE, *Secretary*

PNEUMONOCONIOSIS DEVELOPING AFTER FOURTEEN MONTHS' USE OF A SAND BLAST. F. D. STUBBS and H. M. PAYNE.

For fourteen months, from August 1934 to October 1935, a Negro aged 57 years worked with a sand blast at polishing metal castings. No precautions against inhalation of dust were used, neither a mask nor a suction exhaust being supplied. In October 1935, he gave up his job because of dyspnea, which became worse in the following year and was accompanied by swelling of the feet and legs. One year after the period of exposure to dust he entered a local hospital. At this time six examinations of the sputum disclosed no acid-fast bacilli. An x-ray film of the chest showed the following changes: In the right lung, the upper three fourths of the field was opaque, homogeneous laterally and less dense mesially in areas in the posterior fourth, fifth, sixth and seventh interspaces. In the lower fourth, dense spots and strands obscured the cardiophrenic angle, and the costodiaphragmatic sulcus was filled in and rounded. The upper half of the left pulmonary field was uniformly opaque except at the lower border, while in the lower half scattered light spots occurred. The costodiaphragmatic sulcus was similar to that on the right. The right cardiac border was largely obscured by the pulmonary infiltration. The customary contour of the left ventricle was replaced by a straight line.

A diagnosis of far advanced pulmonary tuberculosis with pneumonoconiosis was made, and the patient was discharged, unimproved, Oct. 17, 1936, after which he became bed-fast because of dyspnea.

On Dec. 5, 1936, he came under our observation. Symptoms indicated pneumonoconiosis with emphysema and failure of the right side of the heart. He died Dec. 12, 1936, twenty-eight months after the first known exposure to silica and after an illness of fourteen months.

At autopsy significant pathologic changes were limited to the chest, except for marked edema of the dependent portions of the body. The pericardial sac

contained 500 cc. of clear straw-colored fluid. The right auricle and the superior vena cava and its tributary veins were distended with semifluid blood. The right auricle and ventricle were hypertrophied and dilated, and the right side of the heart was much larger than the left. No evidence of coronary, valvular or aortic disease was found. The lungs lay free in the pleural cavity, with large emphysematous blebs over their margins. The pleura was smooth and glistening. After fixation by intratracheal injection of Kaiserling's solution I, the lungs were sectioned. The upper lobes and the middle lobe on the right were the site of a firm homogeneous slate-gray consolidation. The apical portions of both lower lobes contained areas of firm consolidation similar to that in the upper lobes, but the greater portion of the lower lobes was emphysematous, with occasional irregular nodules scattered through the tissue. One of the right inferior tracheobronchial lymph nodes contained a small calcified area, but earlier fixation prevented inoculation of animals with material from this focus.

Microscopic studies of the areas of consolidation in the lungs showed diffuse fibrosis with many hyaline fibrotic nodules and no normal lung tissue. Many of the nodules had necrotic centers, and in some of them the necrotic material was basophilic, suggesting beginning calcification. Brown pigment granules occurred peripherally in the nodules. Many homogeneous hyaline strands occurred throughout the sections. Changes typical of tuberculosis were absent, and tubercle bacilli were not found by special stains. Sections of the lower lobe showed dilatation of the alveoli, with numerous focal collections of lymphocytes, large pigment-bearing cells and fibroblasts in the interstitial tissue. The right inferior tracheobronchial node showed calcareocaseous tuberculosis, without histologic evidence of activity.

PRIMARY CARCINOMA OF THE JEJUNUM, 20 CM. FROM THE SUSPENSORY MUSCLE OF TREITZ. FORREST G. BRATLEY.

A white man 34 years of age was admitted to the Presbyterian Hospital on July 31, 1936. Approximately a year prior to this, he was treated for pain in the epigastrium, which was apparently relieved by alkaline powders. His chief complaints were weakness, loss in weight and tarry stools. Otherwise he had been well until six days previous to admission, when he became nauseated and vomited ten times. Physical examination disclosed only slight tenderness in the epigastrium. Occult blood was present in the stools, and secondary anemia was present and persisted in spite of four transfusions over a period of six weeks. Roentgenologic examination of the gastro-intestinal tract revealed nothing except a deformity of the duodenal cap. A diagnosis of bleeding duodenal ulcer was made, and the patient was put on a regimen for ulcer.

Operation revealed an encircling tumor of the jejunum, 20 cm. from the suspensory muscle of Treitz. Ten cubic centimeters of jejunum was resected and an end to end anastomosis performed.

The tumor was fairly well circumscribed, raised, and completely encircled the intestine. At its widest point it measured 2 cm. The lumen of the intestine was constricted to a diameter of 1 cm. Microscopically, the structure was adenocarcinoma, and it had invaded the muscularis. The lymph nodes removed with the resected bowel did not show metastases.

Six months postoperatively the patient was seen in the follow-up clinic, and he had regained his former good health and weight.

ACTIVE IMMUNIZATION AGAINST ACUTE ANTERIOR POLIOMYELITIS WITH RICINOLEATED VACCINE. JOHN A. KOLMER and ANNA M. RULE.

Effective immunization with ricinoleated vaccine requires administration of vaccine containing sufficient living virus to produce paralysis of monkeys when inoculated intracerebrally in amounts of 0.3 cc. Further, we have noted that a Berkefeld filtrate of the ricinoleated vaccine was without immunizing value in 10 monkeys in the doses employed. The supernatant fluid obtained by centrifugating

the vaccine was more antigenic in the immunization of 8 additional animals but not quite as antigenic as the regular vaccine employed in the immunization of 8 animals. The best results were observed, therefore, with a ricinoleated vaccine of very finely divided cord substance.

Of a total of 183 monkeys given subcutaneous or intracutaneous injections of ricinoleated vaccine, 3, or 1.1 per cent, presented paralysis during the period of immunization. All of these were among the 124 animals immunized by subcutaneous injections, and this occurred during the past year, when our strain of virus was of greater virulence than in 1934. None of 59 animals given intracutaneous injections showed any evidences of infection during immunization.

The intracutaneous administration of the vaccine, therefore, is apparently safer than the subcutaneous.

All of a group of 18 animals showed the presence of virus-neutralizing antibody following subcutaneous or intracutaneous injections of ricinoleated vaccine. All were found protected when 0.2 cc. of 5 per cent virus was inoculated intracerebrally, but when 0.5 per cent virus was inoculated eighteen days later 3 presented paralysis.

We believe that the antibody engendered by ricinoleated vaccine plays some rôle in the immunity of monkeys, but the immunity is relative and probably requires large amounts of antibody for the protection of the nervous system following intracerebral and intranasal inoculations of the virus.

Of 80 rhesus monkeys given from five to ten subcutaneous injections of ricinoleated vaccine in doses varying from 0.05 to 1 cc. per kilogram, 61, or 76 per cent, were found completely protected when 0.5 cc. of 5 per cent virus was inoculated intracerebrally, while 17 unvaccinated controls had paralysis within from five to eight days. Among the animals given from 0.05 to 0.4 cc. per kilogram, from 64 to 75 per cent were found protected, while doses of 0.5 and 1 cc. completely protected from 93 to 100 per cent of a group of 21 animals. Of 7 additional monkeys given five subcutaneous injections of vaccine in doses varying from 0.1 to 1 cc. per kilogram, 5 were found protected when 0.5 cc. of a 10 per cent virus was inoculated (two instillations) intranasally two weeks later, while a control showed complete paralysis.

Of 43 animals given from five to ten intracutaneous injections of the vaccine in doses varying from 0.05 to 0.5 cc. per kilogram, 33, or 77 per cent, were found completely protected when the same dose of virus was inoculated intracerebrally, while all of the same 17 unvaccinated controls had paralysis within from five to eight days.

Intracutaneous injections of the vaccine, therefore, engender immunity to a higher degree than subcutaneous injections, since doses of 0.1 and 0.25 cc. per kilogram immunized from 80 to 94 per cent of animals, while similar doses on subcutaneous injection immunized from 73 to 75 per cent.

THE PATHOLOGY OF POLIOMYELITIS. LAWRENCE W. SMITH.

Poliomyelitis presents two distinct pathologic phases. One of these, which appears early in the course of the disease in man, affects the reticulo-endothelial system. The changes representative of this phase are not pathognomonic but are similar to the toxic changes seen in other acute infectious diseases. This phase is characterized by marked hyperplasia of the reticulo-endothelial apparatus, notably affecting the lymphoid tissues of the intestine, the mesenteric lymph nodes, the spleen, the tonsils to a less marked degree and regularly the thymus. The weight of the thymus in poliomyelitis averages from 15 to 20 per cent more than it does in any of the other acute infectious diseases of childhood. The visceral pathologic picture is one of mild toxic degeneration with cardiac dilatation and myocardial changes of a mild grade. Similarly, toxic hepatitis and nephrosis are observed. The liver, grossly, has an almost characteristic old rose discoloration as viewed through the capsule. Another feature of the general pathologic picture of poliomyelitis is petechial hemorrhage in the gastric mucosa

in over a third of the cases. This we attribute to toxic injury to the vascular endothelium and believe that the hemorrhages in the central nervous system might be explained on the same basis.

The other major phase of the pathologic process is seen in the central nervous system, where the virus has a specific neurotropic effect. The portal of entry appears to be particularly the olfactory bulbs, as evidenced by the changes found in these organs at times in man and fairly regularly in the experimental animal. This does not preclude the passage of the virus along any other nerve tracts. The earliest changes in the central nervous system are those involving the pia arachnoid, particularly around the nerve roots. The changes in the central nervous system are histologically identical in development and vary only in degree and distribution. There are initial edema and congestion of the entire central nervous system, which are most marked from the brain stem down. These are followed frequently by petechial hemorrhage. The nerve cell change is one or the other of two types, karyolytic and karyorrhectic. This is accompanied by accumulation of microglia cells, which become transformed into typical scavenger gitter cells, which phagocytize the necrotic ganglion cells. This is entirely apart from the perivascular lymphocytic inflammatory infiltration which is so characteristic of the disease. The neuronophagia of these microglia cells is one of the outstanding diagnostic features of the disease. In the extreme cases, actual liquefaction necrosis of considerable areas of the cord may occur. In healing in late cases definite gliosis has been observed.

The facts recorded here represent data accumulated from the study of post-mortem records in 154 cases of poliomyelitis. The ages of the patients varied from 2 months to 37 years; the duration, from twenty-four hours to nine months. The peak of the mortality occurs on the fifth or the sixth day and corresponds to that of the morbidity.

Book Reviews

Histochimie animale. Méthodes et problèmes. By L. Lison. With a preface by Pol Gérard. Paper. Pp. 320. Price, 50 francs. Paris: Gauthier-Villars, 1936.

In the foreword Gérard points out the lack of a comprehensive account of the results of investigation in the field of histochemistry. Lison has filled this need by writing the first authoritative and exhaustive treatise on the methods and problems of histochemistry—a task for which he is well fitted by virtue of his own investigations and contributions over many years. In the introductory chapters, which are especially worthy of study, he treats of the scope and practicalities of the general methods of histochemistry and those problems which may be solved by microchemistry. Emphasis is placed on the importance of fixation, the corner-stone of histologic technic, in the preliminary preparation of tissues for histochemical tests. He wisely distinguishes between specific histochemical tests, capable of explanation in terms of chemistry or physics or both, and those which are merely color reactions. He draws attention to the importance of the limits of microscopic vision in the interpretation of those histochemical tests which are based on the development of a new compound from the reaction of the substance in the tissue with a reagent. For example, the prussian blue reaction for iron is positive only if the blue-colored iron particles are present in a concentration sufficient to be optically visible. A negative iron test does not necessarily indicate the absence of iron.

A much needed critical survey precedes the discussion of special methods. The pathologist will learn that only few of the so-called classic histochemical tests for organic and inorganic substances in the cells are above suspicion. There is a thorough treatment of the tinctorial, optical, physical and chemical properties of pigments, ferments, lipoids, vitamins, etc., with a critical evaluation of the significance of their demonstration in the cell. The technic of Giroud and Leblond for the detection of vitamin C in tissues is apparently accepted without reservation in spite of the fact that reduction of silver nitrate by vitamin C is interfered with by glutathione, as pointed out by Svirbely. The chapter on fats and allied substances is particularly strong. There is a thorough discussion of the newer tools of investigation, such as micro-incineration and spectrum analysis.

The style has the traditional Gallic clarity. A well selected and useful bibliography is appended to each section. Unfortunately there is no subject index, which mars the usefulness of the book. Such an index should be included in the next edition.

The pathologist will discover in this volume a mine of sound and valuable information. More important, his interest will be stimulated in a field which has lain somewhat fallow in this country in recent years. It should be pointed out that close cooperation and pooling of knowledge between pathologist and chemist are essential for the advancement of histochemistry.

Fluorine Intoxication—A Clinical-Hygienic Study, with a Review of the Literature and Some Experimental Investigations. By Kaj Roholm. Pp. 364, with 96 illustrations. Copenhagen: Nyt Nordøsk Forlag. London: H. K. Lewis & Co., Inc., 1937.

In this study of fluorine poisoning, Roholm treats systematically the rôle played by fluorine in biology, with a careful analysis of the literature. He emphasizes the genesis of the various forms of intoxication and describes in detail their clinical and pathologic features besides presenting an account of the occurrence of fluorine in inanimate and animate nature. The section devoted to human cryolite intoxication from the point of view of the clinical picture, morbid anatomy and industrial hygiene is based on the author's investigations at a cryolite factory in Copenhagen.

His intoxication experiments on rats, pigs, calves and dogs are instructive. Fluorine poisoning is important to the pathologist, the clinician, the veterinarian and the toxicologist because of its prevalence in many countries where substances containing fluorine are accidentally ingested. Endemic discoloration of the teeth due to fluorine-containing drinking water occurs in small restricted regions in Texas, Arizona, Colorado, Mexico, Argentina, the Barbadoes and the Bahama Islands, in the environs of Rome, in limited areas in Spain, England and Holland, in Algeria, Tunisia and Morocco, where large phosphorite deposits are present, in certain parts of South Africa, in the Azores and the Cape Verde Islands and in North China and Japan. The disease usually affects the permanent teeth of children who have grown up in these localities and consists of a spotty loss of the normal translucency, leaving a mottled chalklike appearance as well as irregular or transverse deposits of yellow, brown or black pigment in the defective enamel of the canine and incisor teeth. The histologic appearance is that of defective deposition of interprismatic substance in the enamel and dentin, associated with imperfect calcification of prisms and interprismatic substance. Fluorine poisoning occurs as a dental disease in those engaged in the cryolite industry and as a dental and osseous disease in herbivora in Europe, North Africa and Iceland. The essential causes lie in drinking water containing more than 1 mg. of fluorine per liter, plants cultivated on soil rich in fluorine, fluoric products used in industry and fluoric volcanic products. Among the recommendations for prevention are neutralization of fluoric waste, prohibition of women and young people from working in such concerns and elimination of fluorine from drinking water and food.

Bacteriology of Specific Communicable Diseases. Handbook of Public Health Bacteriology. General Information Regarding Epidemiology, Collection and Shipment of Specimens, and Bacteriologic, Serologic and Chemical Procedures, 1937. Edited by M. S. Marshall. Paper. Pp. 141. San Francisco: J. W. Stacey, Inc., 1937.

The title indicates the nature and purpose of this manual, which is published under the auspices of the department of public health of San Francisco. The reportable diseases in California and the various materials used for examination in the laboratory are listed, with instructions as to the handling and shipment of specimens. The bulk of the book deals instructively with the bacteriologic, serologic and chemical procedures in current use in public health laboratories. The book (coil-bound) is a well arranged, useful guide to practical work in its field.

El groupo indeterminado de las affectiones malignas de los ganglios linfaticos (linfogranulomatosis atipica). By Dr. N. Puente Duany. Pp. 94, with 47 illustrations. Havana, Cuba: A. Sanchez Velosa, 1937.

After a thorough review of the literature the clinical and pathologic courses of ten cases of lymphogranulomatosis are described, in most of which Sternberg giant cells were absent. The tumors closely resembled sarcoma, lymphosarcoma or endothelioma, with occasional metastases in the lymphatics and blood vessels. The possible significance of the tubercle bacillus is considered.

Books Received

La pathogénie des oedèmes. Confrontation des théories à la clinique. Pierre Mauriac, professeur de Clinique médicale, Doyen de la Faculté de médecine de Bordeaux. Paper. Price, 16 francs. Pp. 88. Paris: Masson & Cie, 1937.

Analyse physique des calculs urinaires et biliaires. Le Dr. E. Pillet. Paper. Price, 25 francs. Pp. 96, with 68 illustrations. Paris: Masson & Cie, 1937.

L'hormone folliculaire en physiologie normale et pathologique. Dr. Henri Simonnet, professeur à l'Ecole nationale vétérinaire d'Alfort. Paper. Price, 100 francs. Pp. 532. Paris: Masson & Cie, 1937.

Fluorine Intoxication: A Clinical-Hygienic Study with a Review of the Literature and Some Experimental Investigations. Kaj Roholm. Paper. Pp. 364, with 96 illustrations. Copenhagen: NYT Nordisk Forlag, Arnold Busck, and London: H. K. Lewis & Co., Ltd., 1937.

Christian R. Holmes, Man and Physician. Martin Fischer. Cloth. Price, $4. Pp. 233, with illustrations. Springfield, Ill.: Charles C. Thomas, Publisher, 1937.

Clio Medica Series of Primers on the History of Medicine. Edited by E. B. Krumbhaar. XIX. Pathology. E. B. Krumbhaar, Professor of Pathology, University of Pennsylvania School of Medicine. Cloth. Price, $2. Pp. 206, with 18 illustrations. New York: Paul B. Hoeber, Inc., 1937.

Report of the Penrose Research Laboratory, Formerly Laboratory and Museum of Comparative Pathology, of the Zoological Society of Philadelphia, in Conjunction with the Sixty-Fifth Annual Report of the Society. Herbert Fox, M.D., pathologist. Paper. Pp. 40, with 2 illustrations. 1937.